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The smoking cessation drug varenicline improves deficient P20-N40 inhibition in DBA/2 mice

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Abstract

Varenicline, an FDA approved smoking cessation pharmacotherapy, is an $\alpha 4\beta 2^*$ nicotinic acetylcholine receptor (nAChR) partial agonist and an $\alpha 7^*$ nAChR full agonist. Both subtypes of nAChR are involved in modulating auditory evoked responses in rodents. In DBA/2 mice, an inbred strain, auditory evoked responses to paired auditory stimuli fail to inhibit to the second stimulus. This mouse strain replicates the auditory evoked response inhibition deficit experienced by the majority of schizophrenia patients. In this current study, we examined the effects of five different doses of varenicline (0.06, 0.3, 0.6, 3 and 6 mg/kg) on auditory evoked responses in anesthetized DBA/2 mice. We also examined $\alpha 4\beta 2^*$ and $\alpha 7^*$ nAChR selective antagonist pre-administration prior to varenicline administration in order to determine the impact of these two nAChR subtypes in the effects produced by varenicline. Four of the five doses of varenicline produced improvements in auditory evoked response inhibition deficits. Selective blockade of either the $\alpha 4\beta 2^*$ or $\alpha 7^*$ nAChR in competition with 0.6 mg/kg varenicline prevented varenicline induced improvements. In competition with a higher dose of varenicline (3 mg/kg) only blockade of the $\alpha 4\beta 2^*$ nAChR prevented varenicline induced improvement in auditory evoked response inhibition. These data indicate the importance of $\alpha 4\beta 2^*$ nAChRs and the potential involvement of the $\alpha 7^*$ subtype in varenicline's effects on auditory evoked responses in DBA/2 mice.

Keywords

nicotinic acetylcholine receptors; varenicline; DBA/2 mice; dihydro- β -erythroidine; α -bungarotoxin; sensory inhibition

1. Introduction

The smoking cessation pharmacotherapy, varenicline, is currently the most successful FDA approved drug for achieving abstinence from smoking, as compared to placebo, bupropion and nicotine replacement therapies (Cahill et al., 2008; Gonzales et al., 2006; Jorenby et al., 2006; Nides et al., 2006; Oncken et al., 2006; Tonstad et al., 2006; Williams et al., 2007).

Varenicline is a partial agonist for heteromeric $\alpha 4\beta 2^*$ nicotinic acetylcholine receptors (nAChRs) and full agonist for homomeric $\alpha 7^*$ nAChRs, relative to the endogenous nAChR ligand, acetylcholine (Mihalak et al., 2006; Papke et al., 2010). Varenicline also possesses

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efficacy for $\alpha 3\beta 2$ and $\alpha 3\beta 4$ heteromeric nAChRs, however, its potencies for these receptor subtypes are at least 14-fold less than for the $\alpha 4\beta 2^*$ subtype (Mihalak et al., 2006; Papke et al., 2010). It is proposed that varenicline's clinical efficacy is via mesolimbic dopamine release following nAChR activation. Dopamine release in rodents subsequent to varenicline administration is proposed to occur due to varenicline's interaction with $\alpha 4\beta 2^*$ nAChRs in the ventral tegmental area (Coe et al., 2005; Reperant et al., 2010; Rollema et al., 2007a). Varenicline induced dopamine release alleviates decreased levels of dopamine experienced during nicotine withdrawal. In addition, as an $\alpha 4\beta 2^*$ partial agonist, varenicline inhibits additional dopamine release in the presence of nicotine, therefore reducing the reinforcing effects of nicotine (Coe et al., 2005; Cohen et al., 2003; Rollema et al., 2007b).

Varenicline is a compound of interest in our laboratory as both $\alpha 7^*$ and $\alpha 4\beta 2^*$ nAChRs mediate aspects of sensory inhibition of auditory evoked responses, a measure of sensory processing (Adler et al., 1999; Radek et al., 2006; Stevens et al., 1996; Wildeboer and Stevens, 2008). Among persons diagnosed with schizophrenia, the majority experience deficits in the inhibition of repeated auditory evoked responses (Adler et al., 2004). These deficits manifest as a lack of inhibition of response to repeated auditory stimuli, such that persons report an inability to focus attention and experience a "flooding" of auditory stimuli (Venables, 1964). In humans, these auditory evoked responses are measured by electroencephalograms (EEGs) with a conditioning-test paradigm. Measured neuronal inhibition to repeated auditory stimuli originates in the hippocampus (Grunwald et al., 2003). In response to a pair of auditory stimuli, a positive voltage inflection, termed the P50 waveform, is observed in response to each stimuli. During normal inhibition, decreases in amplitude in response to the second (test) stimulus as compared to the amplitude of the first (conditioning) stimulus are observed. Deficient inhibition, which is a hallmark of schizophrenia, is defined as the ratio of P50 amplitudes (test/conditioning) with a value of 0.5 or greater (Freedman et al., 1997; Leonard et al., 1996). The ratios of test/conditioning amplitudes are referred to as T/C ratios. In humans this deficit has been genetically linked to chromosome 15 locus q13.3 (van Bon et al., 2009), the location of the $\alpha 7$ nAChR gene (CHRNA7). In post-mortem brain tissue from persons with schizophrenia, the level of $\alpha 7^*$ nAChR binding, as determined by radiolabeled alpha-bungarotoxin (BTX), is decreased compared to tissue from non-schizophrenia persons, as is the level of $\alpha 4\beta 2^*$ nAChR binding, as determined by radiolabeled cytosine (Freedman et al., 1995).

In rodents, the P20-N40 waveform complex is analogous to the P50 waveform in humans (Hashimoto et al., 2005). The DBA/2 mouse, an inbred strain, models the auditory evoked response deficit experienced among persons diagnosed with schizophrenia (Stevens et al., 1996). The DBA/2 mouse has endogenously low levels of $\alpha 7^*$ nAChR binding as compared to other mouse strains, such as C57/BL6 with intermediate levels or C3H with levels higher than DBA/2 or C57/BL6 (Marks et al., 1989). Both C57/BL6 and C3H mice, with higher levels of $\alpha 7$ binding, exhibit normal auditory evoked responses whereas low levels of $\alpha 7^*$ binding observed for DBA/2 mice has been correlated with the auditory evoked response deficit (Stevens et al., 1996). In both humans and rodents expressing the phenotype for deficient inhibitory auditory evoked responses, nicotine transiently improves the deficit (Adler et al., 1993; Stevens and Wear, 1997), as does the selective $\alpha 7^*$ nAChR partial agonist, DMXB-A (Olincy et al., 2006; Stevens et al., 1998). Rodent studies also suggest a role for the $\alpha 4\beta 2^*$ nAChR in modulation of auditory evoked responses (Radek et al., 2006; Rudnick et al., 2009). Agonists for $\alpha 4\beta 2^*$ nAChRs produce improvements in the deficient phenotype (Stevens and Wear, 1997; Wildeboer and Stevens, 2008). It had been previously determined that DMXB-A produces improvements in the auditory evoked response deficit of DBA/2 mice by decreasing the amplitude of the test response (Simosky et al., 2001; Stevens et al., 1998), whereas an agonist for $\alpha 4\beta 2^*$, 5-I 85380, produces improvements by increasing the amplitude of the conditioning response (Wildeboer and Stevens, 2008). Thus,

varenicline, with its dual receptor agonist profile, was tested for its potential impact in our DBA/2 mouse model for deficient inhibition of repeated auditory evoked responses. In this study, we examined the effect of varenicline in male DBA/2 mice alone and following pre-administration of selective $\alpha 4\beta 2^*$ or $\alpha 7^*$ nAChR antagonists. Based on previously published literature of varenicline's interaction with $\alpha 4\beta 2^*$ nAChRs (Coe et al., 2005; Mihalak et al., 2006; Papke et al., 2010; Reperant et al., 2010; Rollema et al., 2007a), we hypothesized that varenicline would transiently improve DBA/2 deficient auditory evoked responses and that the effects would be produced primarily via $\alpha 4\beta 2^*$ nAChRs.

2. Materials and Methods

2.1 Animals

Male DBA/2 mice (20-25 g) were obtained from Harlan Sprague Dawley (Indianapolis, IN) and housed five to a cage in ventilated cage racks at the Center for Comparative Medicine at the University of Colorado Denver, School of Medicine (UCD-SOM). The mice were provided water and food (Harlan Teklad, Indianapolis, IN) *ad libitum*. Lighting was cycled at 12 hour intervals with lights on at 6:00 AM. All animal procedures were approved by the Institutional Animal Care and Use Committee of UCD-SOM and conform to the Principles of Laboratory Animal Care (Institute of Laboratory Animal Resources, 1996).

2.2 Surgery

For electrophysiological recording of auditory evoked responses, mice were anesthetized by intraperitoneal (IP) injection of the anesthetic chloral hydrate (400 mg/kg) followed by an injection (IP) of pyrazole (400 mg/kg) to retard the metabolism of chloral hydrate. Once a surgical plane of anesthesia was attained, mice were placed in a Kopf stereotaxic instrument (Kopf Instruments, Tujunga, CA) on a heating pad (35° C) to maintain a stable core temperature. Hollow earbars, attached to miniature earphones connected to an audio amplifier, were placed adjacent to the externalization of the aural canal. During recording, chloral hydrate and pyrazole were supplemented as necessary (5 mg/kg, IP) to maintain a surgical plane of anesthesia as evidenced by lack of reflexive limb withdrawal in response to toe pinch.

The scalp was incised and burr holes drilled over the dorsal CA3 region of the hippocampus [−1.8 mm posterior from bregma, +2.5 mm lateral from midline (Paxinos and Franklin, 2001) and the contralateral anterior cortex. The recording electrode, a Teflon-coated stainless-steel cut wire (0.127 mm diameter), was inserted into the CA3 pyramidal cell layer of the hippocampus (1.5 to 1.7 mm ventral from the dorsal brain surface). Final placement of the recording electrode was determined by the presence of complex action potentials typical of hippocampal pyramidal neurons (Miller et al., 1992). The reference electrode, identical in composition to the recording electrode, was placed on dura through the burr hole over the contralateral cortex. Antagonist experiments involving dihydro- β -erythroidine (DH β E) or α -bungarotoxin (BTX) required a third burr hole to be drilled over the anterior lateral ventricle (+0.8 mm anterior from bregma, −0.5 mm lateral from midline (Paxinos and Franklin, 2001)) ipsilateral to the recording electrode for placement of an injection cannula. A 26-gauge needle attached to a 10 μ l Hamilton syringe (Hamilton, Reno, NV) was inserted into the anterior lateral ventricle (2.0 mm below the dura) for intracerebroventricular (ICV) administration of antagonist.

2.3 Experimental Protocol

Tones (3000 Hz, 10 ms, 70 dB), as the auditory stimuli, were presented in pairs separated by a 500 millisecond interval with 10 seconds between pairs of stimuli. Responses to 16 pairs of tones were filtered with a bandpass between 10 and 5000 Hz. The N40 wave was defined

as the maximum negativity between 20 and 60 milliseconds after stimulus onset and measured relative to the proceeding positivity, the P20 wave. The measure of the animals' auditory inhibition is reported as a T/C ratio. The T/C ratio is defined as the ratio of the amplitudes of the response to the second tone, the test amplitude, to the response to the first tone, the conditioning amplitude. A decrease in T/C ratio after drug administration, as compared to pre-drug baseline indicates improved inhibition of auditory processing. Five baseline records, at 5-minute intervals, were obtained prior to compound administration. Previous studies in our lab confirmed that vehicle control injections had no impact upon T/C ratios in DBA/2 mice (Hashimoto et al., 2005; Stevens and Wear, 1997). Electrical responses were amplified 1000 times with analog to digital conversion (SciWorks, DataWave, Loveland, CO) and averaged by computer. Data were collected, stored and analyzed with the SciWorks computer program (DataWave, Loveland, CO).

Varenicline was dissolved in 0.9% NaCl and administered IP at five doses (0.06 mg/kg, $n = 6$; 0.3 mg/kg, $n = 11$; 0.6 mg/kg, $n = 12$; 3 mg/kg, $n = 11$; 6 mg/kg, $n = 11$). After injection, recordings continued for up to 40 minutes, at 5 minute intervals.

For antagonist experiments, either 27 nM DH β E or 1.25 nM BTX was administered at a volume of 1 μ l into the anterior lateral ventricle following baseline recordings (Simosky et al., 2003). After injection of antagonist, four records at 5 minute intervals were obtained to verify that the antagonist alone did not affect TAMP, CAMP or T/C ratio. After these four records of antagonist alone, an injection of either 0.6 mg/kg or 3 mg/kg IP varenicline was administered. Records at 5-minute intervals were obtained for 40 minutes of records, thereafter.

2.4 Compounds

Varenicline tartrate was graciously provided by Pfizer (Groton, CT), BTX and DH β E hydrobromide were obtained from Tocris (Ellisville, MO). Varenicline, DH β E and BTX doses were calculated as free base weight. All compounds were dissolved in 0.9% NaCl.

2.5 Statistical analysis

The time course of varenicline alone or in conjunction with antagonist was analyzed, for each dose, using repeated measures MANOVA. A significant p-value ($p < 0.05$) obtained from a MANOVA was followed by Fisher's protected least-significant difference (PLSD) *a posteriori* analysis to compare individual post injection time points to collapsed average baseline values.

3. Results

Varenicline was administered to DBA/2 mice at five different doses (0.06, 0.3, 0.6, 3 and 6 mg/kg). All but one of the doses tested (0.06 mg/kg) produced improvements in inhibitory processing of the P20-N40 auditory evoked response. The improvements were evidenced by significant decreases in the T/C ratios (Figure 1), defined as the ratio of amplitudes of the evoked potential of the test response to that of the conditioning response (Figure 2). Of the five doses tested, 0.3, 0.6, 3 and 6 mg/kg IP produced significant changes on T/C ratio [0.3 mg/kg: $F_{(13,130)} = 2.27$, $p = 0.010$; 0.6 mg/kg: $F_{(13,143)} = 3.22$, $p < 0.001$; 3 mg/kg: $F_{(13,130)} = 3.26$, $p < 0.001$; 6 mg/kg: $F_{(13,130)} = 2.98$, $p = 0.001$]. Fisher's PLSD *a posteriori* analysis revealed multiple time points, at each dose, with significant decreases in T/C ratio, at least 10 minutes following varenicline administration, relative to the average of baseline measurements (Figure 1).

Analysis of the conditioning and test amplitudes indicated that there were significant changes in both parameters, depending on the dose of varenicline administered (Figure 1).

At the second lowest dose, 0.3 mg/kg (IP), there was no statistically significant effect on either the conditioning or test amplitudes. However, the conditioning amplitude did trend towards an increase as compared to baseline following varenicline administration [$F_{(13,130)} = 1.76$, $p = 0.056$]. The 0.6 mg/kg dose produced a significant effect on the test amplitude [$F_{(13,143)} = 2.24$, $p = 0.011$]. Fisher's PLSD *a posteriori* analysis revealed significant decrease in the test amplitude at the 10 and 15 minute time points following varenicline administration. There was no significant effect on the conditioning amplitude at this dose. The 3 mg/kg dose of varenicline produced no effect on the test amplitude whereas there was a significant effect on conditioning amplitude [$F_{(13,130)} = 2.88$, $p = 0.001$]. Fisher's PLSD *a posteriori* analysis showed that the 5 and 10 minute time points following varenicline administration were significantly increased. The highest dose of varenicline tested, 6 mg/kg (IP), produced no significant effect on the test amplitude but again significantly impacted the conditioning amplitude [$F_{(13,130)} = 3.02$, $p = 0.001$]. Fisher's PLSD *a posteriori* analysis detected significant increases in conditioning amplitude at the 15, 25, 30 and 40 minute time points following varenicline administration.

Although varenicline is proposed to work via the $\alpha 4\beta 2^*$ nAChR subtype, it also has agonist activity at the $\alpha 7^*$ subtype (Mihalak et al., 2006; Papke et al., 2010). In order to determine the relative impact of activation of either $\alpha 4\beta 2^*$ or $\alpha 7^*$ nAChRs following varenicline administration, selective antagonists for either receptor subtype were centrally administered 20 minutes prior to the injection of varenicline but following five baseline recordings. Interestingly, antagonism of the $\alpha 7^*$ subtype by BTX (1.25 nM, ICV) also blocked varenicline (0.6 mg/ml) induced improvement in T/C ratio compared to administration of varenicline alone (Figure 3). Blockade of the $\alpha 4\beta 2^*$ subtype by DH β E (27 nM, ICV) prevented significant decreases in T/C ratio following varenicline administration (0.6 mg/kg, IP) (Figure 4). Antagonism of each receptor subtype was also examined in competition with 3 mg/kg varenicline in order to determine a possible concentration dependent mechanism of varenicline at each receptor subtype. At the 3 mg/kg dose of varenicline, the $\alpha 7^*$ receptor antagonist (BTX) failed to prevent varenicline induced significant decreases in T/C ratio (Figure 5). Antagonism of the $\alpha 4\beta 2^*$ subtype by DH β E (27 nM, ICV) prevented significant decreases in T/C ratio following 3 mg/kg varenicline administration (Figure 6), similar to the 0.6 mg/kg varenicline dose (Figure 4). There were no significant changes in either the test or conditioning amplitude following antagonist plus varenicline administration for either BTX or DH β E (Figures 3, 4, 5 and 6).

4. Discussion

The findings of our study indicate increased inhibition of auditory evoked responses to repeated auditory stimuli and overall improvement of P20-N40 inhibition in DBA/2 mice with administration of varenicline. Auditory evoked responses, as a measure of sensory processing, are proposed to be a pre-attentional aspect of early sensory processing (Rollema et al., 2009) or the habituation of the auditory evoked responses to repeated stimuli (Rudnick et al., 2010). In rats expressing normal auditory evoked responses, varenicline does not impact P20-N40 inhibition (Rollema et al., 2009). However, in rats with amphetamine induced deficits in P20-N40 inhibition, varenicline transiently reduced the deficit recorded in the CA3 region of the hippocampus, indicating modulation of auditory evoked responses via $\alpha 4\beta 2^*$ nAChRs (Rollema et al., 2009). Our results in DBA/2 mice are consistent with the previous finding that varenicline improves P20-N40 inhibition in a deficient model.

Whereas previous reports indicate a significant role of $\alpha 4\beta 2^*$ nAChRs in varenicline's mechanism of action (Rollema et al., 2009; Rudnick et al., 2010), our results suggest a possible role for $\alpha 7^*$ nAChRs in varenicline induced improvement of deficient P20-N40 inhibition, via modulation of the test amplitude, of DBA/2 mice. At 0.6 mg/kg, varenicline

significantly decreased the test amplitude with no significant effect on the conditioning amplitude (Figure 1). A significant effect on the test amplitude is thought to be mediated via $\alpha 7^*$ nAChRs (Stevens et al., 1998) whereas a significant effect on the conditioning amplitude is proposed to occur via $\alpha 4\beta 2^*$ nAChRs (Radek et al., 2006; Wildeboer and Stevens, 2008). Thus, varenicline may be activating $\alpha 7^*$ nAChRs at 0.6 mg/kg in our DBA/2 mouse model. Although varenicline is a full agonist for $\alpha 7^*$ nAChRs, previous studies quantify the affinity of varenicline to be much greater for the $\alpha 4\beta 2^*$ subtype as compared to the $\alpha 7^*$ subtype (Rollema et al., 2009). In addition, the functional EC_{50} value of varenicline is higher for $\alpha 7^*$ than for $\alpha 4\beta 2^*$ (Mihalak et al., 2006). A recent study examined the effect of varenicline on mouse $\alpha 4\beta 2$ and $\alpha 7$ nAChRs expressed in *Xenopus* oocytes. The EC_{50} of varenicline for the C57BL/6 mouse $\alpha 7$ was 0.8 μM as compared to 2.6 μM for C57BL/6 $\alpha 4\beta 2$ (Papke et al., 2010). These results suggest that $\alpha 7$ nAChRs may be functionally activated at a lower dose of varenicline than $\alpha 4\beta 2$ in mice. However, it should be noted that the EC_{50} value determinations of varenicline on mouse nAChRs were performed on C57BL/6 mouse $\alpha 4$ subunits expressed in conjunction with $\beta 2$ nAChR subunits in *Xenopus* oocytes (Papke et al., 2010). This mouse strain expresses the more common functional genetic variant of the $\alpha 4$ nAChR subunit, a threonine at position 529 rather than an alanine (DBA/2 mice) (Dobelis et al., 2002; Kim et al., 2003; Stitzel et al., 2001). Because the functional responses of these two variants differ, it is possible that the functional response of DBA/2 mice to varenicline may differ from those of C57BL/6 mice.

Previously, the effect of varenicline on auditory evoked responses of C57BL/6 mice was determined to produce an overall increase in P20 amplitude in response to both the first and second auditory stimuli (Rudnick et al., 2010). This study examined only the peak response, P20, and not the entire P20-N40 waveform. Because they observed increased P20 amplitudes to both the first and second stimuli, they did not observe an effect on sensory inhibition. Our results demonstrate that varenicline produces a significant decrease in T/C ratio, indicating an improvement in P20-N40 inhibition. Differences in outcome between the current and former study are most likely due to several factors, including the difference in mouse strains, and differences in recording and analysis methods. Our study examined an anesthetized P20-N40 deficient model whereas the former study examined a model of normal auditory evoked response in the non-anesthetized state. The present study assessed the full P20-N40 wave complex, which has been found to be less variable than either individual component (Hashimoto et al., 2005) while the Rudnick et al. (2010) study only assessed the P20 component.

In order to further examine the involvement of $\alpha 7^*$ and $\alpha 4\beta 2^*$ nAChRs in varenicline's action on P20-N40 inhibition, we administered selective antagonists prior to administration of varenicline. Selective antagonists for either $\alpha 7^*$ or $\alpha 4\beta 2^*$ have been reported to produce no effects on the test or conditioning amplitudes or T/C ratio in DBA/2 mice (Simosky et al., 2003). In competition with 0.6 mg/kg varenicline, both $\alpha 7^*$ and $\alpha 4\beta 2^*$ selective antagonists independently prevented any improvements in P20-N40 inhibition, as noted by the lack of significant decrease of T/C ratio (Figures 3 and 4). The antagonist data indicates that at 0.6 mg/kg varenicline, antagonism of either $\alpha 7^*$ or $\alpha 4\beta 2^*$ nAChRs prevents varenicline induced improvements in P20-N40 inhibition in DBA/2 mice. This data suggests that in a deficient P20-N40 endophenotype model, both receptor subtypes may be involved in the effect of varenicline at a low dose (0.6 mg/kg). In competition with 3 mg/kg varenicline, only the $\alpha 4\beta 2^*$ selective antagonist prevented improvement in P20-N40 inhibition (Figure 6). The $\alpha 7^*$ selective antagonist failed to prevent improvement in P20-N40 inhibition following 3 mg/kg varenicline administration, as noted by significant decreases of T/C ratios (Figure 5). These results indicate a possible dose dependent activation of either receptor subtype by varenicline, such that $\alpha 4\beta 2^*$ nAChRs are involved in varenicline induced responses at

higher doses and $\alpha 7^*$ nAChRs may be involved in mediating responses to lower doses of varenicline.

The effect of varenicline on T/C ratio was relatively long lasting with effects observed 25-40 minutes following administration. At 6 mg/kg varenicline, it is possible that other nAChR subtypes, including $\alpha 3\beta 4$, are being activated. It is also possible that at the higher varenicline doses or at the later time points measured, $\alpha 4\beta 2^*$ and $\alpha 7^*$ nAChRs are desensitized. Both a brief exposure to a high concentration of agonist as well as prolonged exposure of a nicotinic agonist can lead to desensitization of the receptors (Colquhoun and Ogden 1988; Papke and Thinschmidt, 1998; Fenster et al. 1999; Gentry and Lukas 2002; Katz and Thesleff, 1957; Vann et al. 2006). Desensitization of nAChRs, as one conformational state of the protein, may present as a lack of inhibition to repetitive auditory stimuli in our paradigm. This would be reflected as an increase in T/C ratio. Although there are significant decreases in T/C ratio at later time points for the 3 and 6 mg/kg doses, we cannot rule out downstream processes following nAChR activation and desensitization. It is possible that dopamine release, following varenicline induced activation of nAChRs on dopaminergic neurons, could also be modulating auditory evoked responses (Coe et al., 2005; Reperant et al., 2010; Rollema et al., 2007a).

Whereas activation of $\alpha 7^*$ nAChRs result in inhibition to the second auditory stimulus (Stevens et al., 1996), activation of $\alpha 4\beta 2^*$ may increase a pre-attentional state with the increase of the conditioning amplitude following the first auditory stimulus. The $\alpha 4\beta 2^*$ subtype has been observed in the molecular layer of the dentate gyrus (Clarke et al., 1985; Pauly et al., 1989). The mossy fiber axons of dentate granule cells synapse onto both pyramidal neurons and interneurons in the CA3 region of the hippocampus (Henze et al., 2000). Therefore, it is possible that varenicline acting on $\alpha 4\beta 2^*$ nAChRs within the dentate gyrus, may alter conditioning amplitude responses within the hippocampal CA3 region. In addition, $\alpha 4\beta 2^*$ nAChRs on neurons within the VTA, which projects diffusely to the dentate gyrus (Clarke et al., 1985; Swanson et al., 1987; Pauly et al., 1989) may cause activation of neurons in dentate gyrus resulting in increase of the conditioning amplitude. The $\alpha 7^*$ subtype are reported to be located on inhibitory interneurons in various regions of the hippocampus as well as in the dentate gyrus, which are involved in the sensory inhibition response (Gray et al., 1996; Alkondon et al., 1999). Activation of this subtype, by varenicline or other agonist, ultimately inhibits pyramidal neuron response to the repeated auditory stimuli, via GABA release which activates GABA receptors on excitatory inputs to pyramidal neurons (Alkondon et al., 1999; Alkondon and Albuquerque, 2001). The inhibition of pyramidal neuron response to repetitive auditory stimuli, following $\alpha 7^*$ nAChR activation, is reflected in decreased test amplitudes.

There are possible limitations for the use of varenicline in persons with a diagnosed mental illness. Recent reports reveal a potential for a varenicline-induced exacerbation of psychiatric symptoms in patients with a diagnosed mental illness, including major depressive disorder, depression, bipolar disorder, attention deficit hyperactivity disorder, post-traumatic stress disorder and schizophrenia. The side effects produced in this population include visual hallucinations, mania, psychosis, agitation, anxiety, depression and suicidal ideation (Alhatem and Black, 2009; DiPaula and Thomas, 2009; Freedman, 2007; Kohen and Kremen, 2007; Morstad et al., 2008; Popkin, 2008; Pumariega et al., 2008; Raidoo and Kutscher, 2009; Spirling et al., 2008). In contrast to these cases, other published reports indicate no exacerbation of psychiatric symptoms in persons with a diagnosed mental illness (Evins and Goff, 2008; Fatemi, 2008; McClure et al., 2010; Ochoa, 2009).

5. Conclusions

In conclusion, our study provides support for the involvement of $\alpha 4\beta 2^*$ nAChRs, and a possible role for the $\alpha 7^*$ subtype, in varenicline induced improvement of P20-N40 inhibition in DBA/2 mice. Whereas prior studies examined the importance of $\beta 2$ -containing nAChRs in varenicline induced improvements on normal sensory inhibition, our study examined mice deficient for sensory inhibition and also determined that the functional activity of varenicline on $\alpha 4\beta 2^*$ nAChRs produces improvements in sensory inhibition. This study is the first to administer selective nAChR antagonists prior to varenicline administration to examine specific involvement of $\alpha 4\beta 2^*$ and $\alpha 7^*$ nAChRs in the effect of varenicline on P20-N40 inhibition. Because of the genetic association between the $\alpha 7^*$ nAChR and sensory inhibition in persons with schizophrenia, it is important to understand the effect of this drug in the DBA/2 mouse model.

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Research Highlights

>Varenicline is a partial agonist of $\alpha 4\beta 2$ and full agonist of $\alpha 7$ nicotinic receptors.>Varenicline improves a sensory inhibition deficit in DBA/2 mice.>Improvement is via activation of $\alpha 4\beta 2$ with an indication of $\alpha 7$ involvement.

Figure 1. Conditioning and test amplitudes (left) and T/C ratios (right) before and after varenicline administration at four different doses. Varenicline injection (IP) occurred at the dotted line. Records were obtained at 5 minute intervals following injection. Varenicline produced improvements in P20-N40 inhibition at each dose tested as indicated by the significant decreases in T/C ratios (left) as compared to baseline values. Asterisks indicate time points following varenicline administration where a significant effect, as determined by Fisher's PLSD, on either the conditioning amplitude (upper asterisks) or test amplitude (lower asterisks) were found as compared to an averaged baseline for the corresponding dose. Data are mean $\pm$ SEM. * $p < 0.05$, ** $p < 0.01$

Figure 2. Representative P20-N40 waveforms averaged from an individual DBA/2 mouse. Waveforms obtained prior to drug administration (Baseline) and after 0.6 mg/kg varenicline administration. Varenicline administration (0.6 mg/kg, IP) resulted in a decreased T/C ratio indicating an improvement in P20-N40 inhibition. Tick marks denote the waveform and arrows indicate audio stimulus onset. The noise at the start of the waveform represents the audio stimulus artifact. Calibration 20 μ V/10 ms.

Figure 3. Inhibition of varenicline (0.6 mg/kg, IP) induced improvement of P20-N40 inhibition by BTX (1 μ l of 1.25 nM, ICV). The first five time points (baseline) refer to baseline recordings obtained prior to varenicline injection (0.6 mg/kg, IP). Injection of the α 7 antagonist BTX (dotted line) occurred 20 minutes prior to injection of varenicline (dashed line). (●) Represent conditioning amplitude values and (Δ) represent the test amplitude values at each time point. Data are mean $\pm$ SEM; $n = 8$.

Figure 4.

Inhibition of varenicline (0.6 mg/kg, IP) induced improvement of P20-N40 inhibition by DHβE (1 μl of 27 nM, ICV). The first five time points (baseline) refer to baseline recordings obtained prior to drug injection. Injection of the $\alpha 4\beta 2$ antagonist DHβE (dashed line) occurred 20 minutes prior to injection of varenicline (dotted line). (●) Represent conditioning amplitude values and (Δ) represent the test amplitude values at each time point. Data are mean $\pm$ SEM; $n = 11$.

Figure 5.

Inhibition of varenicline (3 mg/kg, IP) induced improvement of P20-N40 inhibition by BTX (1 μ l of 1.25 nM, ICV). The first five time points (baseline) refer to baseline recordings obtained prior to varenicline injection. Injection of the $\alpha 7$ antagonist BTX (dotted line) occurred 20 minutes prior to injection of varenicline (dashed line). (●) Represent conditioning amplitude values and (Δ) represent the test amplitude values at each time point. Data are mean $\pm$ SEM; $n = 9$.

Figure 6. Inhibition of varenicline (3 mg/kg, IP) induced improvement of P20-N40 inhibition by DHβE (1 μl of 27 nM, ICV). The first five time points (baseline) refer to baseline recordings obtained prior to drug injection. Injection of the $\alpha 4 \beta 2$ antagonist DHβE (dashed line) occurred 20 minutes prior to injection of varenicline (dotted line). (●) Represent conditioning amplitude values and (Δ) represent the test amplitude values at each time point. Data are mean $\pm$ SEM; $n = 10$.