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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 α4β2* nicotinic acetylcholine receptor (nAChR) partial agonist and an α7* 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 administered α4β2* and α7* nAChR selective antagonists prior to varenicline administration to determine which nAChR subtypes mediate the effects of varenicline. Four of the five doses of varenicline produced improvements in auditory evoked response inhibition deficits. Selective blockade of either the α4β2* or α7* 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 α4β2* nAChR prevented varenicline induced improvement in auditory evoked response inhibition. These data indicate the importance of α4β2* nAChRs and the potential involvement of the α7* subtype in varenicline's effects on auditory evoked responses in DBA/2 mice.

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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 α4β2* nicotinic acetylcholine receptors (nAChRs) and full agonist for homomeric α7* nAChRs, relative to the endogenous nAChR ligand, acetylcholine (Mihalak et al., 2006; Papke et al., 2010). Varenicline also possesses efficacy for α3β2 and α3β4 heteromeric nAChRs, however, its potencies for these receptor subtypes are at least 14-fold less than for the α4β2* 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 α4β2* 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 α4β2* 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 α7* and α4β2* 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 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

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of the α7 nAChR gene (CHRNA7). In post-mortem brain tissue from persons with schizophrenia, the level of α7* nAChR binding, as determined by radiolabeled alpha-bungarotoxin (BTX), is decreased compared to tissue from non-schizophrenia persons, as is the level of α4β2* nAChR binding, as determined by radiolabeled cytisine (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 inhibition deficit experienced among persons diagnosed with schizophrenia (Stevens et al., 1996). The DBA/2 mouse has endogenously low levels of α7* nAChR binding as compared to other mouse strains, such as C57BL/6 with intermediate levels or C3H with levels higher than DBA/2 or C57BL/6 (Marks et al., 1989). Both C57BL/6 and C3H mice, with higher levels of α7 binding, exhibit normal auditory evoked responses whereas low levels of α7* binding observed for DBA/2 mice has been correlated with the auditory evoked response inhibition 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 α7* nAChR partial agonist, DMXB-A (Olincy et al., 2006; Stevens et al., 1998). Rodent studies also suggest a role for the α4β2* nAChR in modulation of auditory evoked responses (Radek et al., 2006; Rudnick et al., 2009). Agonists for α4β2* 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 inhibition 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 α4β2*, 5-I A-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 α4β2* or α7* nAChR antagonists. Based on previously published literature of varenicline's interaction with α4β2* 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 response inhibition and that the effects would be produced primarily via α4β2* 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 and Freedman, 1995). 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 (DHβE) 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 of electrode for placement of an injection cannula. A 26-gage needle attached to a 10 μl 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 evoked response 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 min, at 5 minute intervals.

For antagonist experiments, either 27 nM DHβE or 1.25 nM BTX was administered at a volume of 1 μl 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 the test amplitude, the conditioning amplitude 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 DHβE hydrobromide were obtained from Tocris (Ellisville,