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Varenicline Ameliorates Nicotine Withdrawal-Induced Learning Deficits in C57BL/6 Mice

Jonathan D. Raybuck[1,2], **George S. Portugal**[2], **Caryn Lerman, Ph.D.**[3], and **Thomas J. Gould, Ph.D.**[1,2,*]

1*Center for Substance Abuse Research, Temple University, Philadelphia, PA 19122*

2*Department of Psychology, Temple University, Philadelphia, PA 19122*

3*Department of Psychiatry and Abramson Cancer Center, University of Pennsylvania, Philadelphia PA (CL)*

Abstract

Varenicline a partial agonist for α4β2 nicotinic acetylcholine receptors (nAChRs) and full agonist for α7 nAChRs, has been approved for the treatment of smoking cessation. While recent clinical trials support the efficacy of varenicline for managing global nicotine withdrawal symptoms and for smoking cessation, varenicline effects on specific withdrawal-associated behaviors in animal models have not been tested. In mice and humans, withdrawal from chronic nicotine disrupts cognitive processing; in mice, this can be measured by changes in contextual fear conditioning. To elucidate potential mechanisms underlying the clinical efficacy of varenicline, the present study evaluated the effects of varenicline on contextual fear conditioning when administered alone and when administered 24 hours after withdrawal from chronic nicotine administration (6.3 mg/kg/day). Varenicline (0.01, 0.1, 1.0 mg/kg) had no effect on contextual fear conditioning when administered alone. However, varenicline dose-dependently prevented nicotine withdrawal-associated deficits in contextual fear conditioning. These data demonstrate that varenicline reverses nicotine withdrawal-induced deficits in an animal model and suggest that varenicline may be effective at treating nicotine withdrawal-associated deficits in learning and memory.

Keywords

Nicotine; Withdrawal; Hippocampus; Fear Conditioning; Addiction; Acetylcholine; Cognition

Introduction

As the leading preventable cause of death in the United States (CDC, 2005), cigarette smoking has dire consequences to both individuals and society. In the United States, over 435,000 deaths are attributed to smoking annually (Mokdad et al., 2004) and the combined direct and indirect economic costs of cigarette smoking are estimated to be 138 billion dollars per year (Rice, 1999). Until recently, the only available FDA-approved pharmacotherapies for smoking cessation included nicotine replacement therapies (NRTs) and bupropion (Frishman et al., 2006; Wu et al., 2006). Varenicline, a partial agonist for α4β2 nicotinic acetylcholine receptors (nAChRs) and full agonist for α7 nAChRs (Mihalak et al., 2006) is the newest FDA-approved

*Corresponding Author, Thomas J. Gould, Ph.D., Department of Psychology, Weiss Hall, Temple University, Philadelphia, PA 19122, Tel: (215) 204-7495, Fax: (215) 204-5539, Email: tgould@temple.edu

Disclosure/Conflict of Interest

Dr. Lerman has served as a paid consultant for pharmaceutical companies, including Pfizer, GlaxoSmith Kline, and Astra Zeneca, on topics unrelated to the present study. The other authors report no conflicts of interest.

medication. Clinical trial data document its superiority to bupropion and NRTs, with quit rates of 50% at the end of treatment and 35% at 6-month follow-up (Gonzales et al., 2006; Jorenby et al., 2006). However, even with pharmacotherapy and counseling, the vast majority of smokers who make a quit attempt will relapse (Schnoll and Lerman, 2006). An increased understanding of the mechanisms through which varenicline facilitates smoking cessation could provide information valuable to medication development for nicotine dependence.

Withdrawal from nicotine, the chemical responsible for tobacco dependence (Benowitz, 1988; DHHS, 1987) produces multiple negative symptoms, including sleep disturbances, negative affect, increased appetite, and cravings (Hughes et al., 1994; Jarvis, 2004), and varenicline can reduce the severity of these symptoms (Gonzales et al., 2006; Jorenby et al., 2006; Nakamura et al., 2007). Additional studies have established that alterations of cognition, including difficulty in concentration (Hughes et al., 1991; Hughes et al., 1994), impairments in attention (Rukstalis et al, 2005; Jacobsen et al., 2005), and deficits in learning and memory (Jacobsen et al., 2005; Mendrek et al., 2006; Jacobsen et al., 2007) also occur in humans during nicotine withdrawal. Importantly, these symptoms can increase the risk of relapse in smokers who attempt to quit (Rukstalis et al, 2005). However, no studies to date have investigated the effects of varenicline on nicotine withdrawal-related deficits in cognition.

Although current research in humans has not addressed the effects of varenicline on nicotine withdrawal-associated deficits in cognition, animal models have been used effectively to evaluate the effects of other smoking cessation drugs (Lerman et al, 2007). One such animal model is Pavlovian fear conditioning, in which a conditioned stimulus (CS) is paired with an aversive unconditioned stimulus (US). Subjects trained in this procedure acquire two associations: an association between the training context and the US (contextual conditioning), and an association between the CS and US (cued conditioning). Research has demonstrated that acute nicotine enhances contextual conditioning, but not cued conditioning (Gould and Wehner, 1999; Gould and Higgins, 2003; Wehner et al., 2004). Conversely, withdrawal from chronic nicotine administration, at a dose comparable to that reported in human smokers (Benowitz et al., 1989; Henningfield and Keenan, 1993; Davis et al., 2005), produces deficits in contextual conditioning but does not affect cued conditioning (Davis et al., 2005; Davis and Gould, 2007; Davis et al., 2007; Portugal and Gould, 2007; Portugal et al., 2008). Nicotine withdrawal-related deficits in contextual conditioning can be ameliorated by nicotine replacement (Davis et al., 2005), by the norepinephrine and dopamine reuptake inhibitor bupropion (Portugal and Gould, 2007), and by the norepinephrine reuptake inhibitor atomoxetine (Davis and Gould, 2007).

As no studies have reported the effects of varenicline on withdrawal-associated cognitive deficits or the effects of varenicline in an animal model of nicotine withdrawal, the goal of the present study was to examine the effects of varenicline on nicotine withdrawal-induced deficits in contextual conditioning in C57BL/6 mice. Additional experiments evaluated whether varenicline administered only on training day or testing day would ameliorate nicotine withdrawal-associated deficits in contextual conditioning.

Methods

Subjects

Male C57BL/6 mice were trained at 8-12 weeks of age (n =7-12). Mice were provided with *ad libitum* access to food and water, maintained on a 12 hour light-dark cycle (lights on at 7:00am), and housed in groups of two or four. Behavioral procedures occurred during the light phase of the cycle. All behavioral and surgical procedures were approved by the Temple University Institutional Animal Care and Use Committee.

Apparatus

The training and testing of contextual fear conditioning was conducted in four identical conditioning chambers (Med-Associates, St. Albans, VT). The chamber floors were composed of 18 stainless steel bars, connected to a shock generator and scrambler (Med-Associates), through which a 2 second 0.57 mA footshock (US) was administered. Speakers attached to the right wall of each chamber provided an 85 dB white noise (CS). Ventilation fans, which provided background noise (69 dB), were mounted on the right wall of each sound-attenuating box. All stimulus administrations were controlled by a computer running Med-PC software (Med-Associates).

Behavioral Procedures: Contextual Fear Conditioning

During training and testing mice were observed for freezing at 10-second intervals (Gould and Wehner, 1999). Freezing was defined as the absence of all movement except for respiration (Blancard and Blanchard, 1969). Training began with the activation of a house light. Baseline activity was scored for 120 s. Next, two co-terminating CS-US pairings, separated by a 120 s inter-trial interval, were presented. CS presentation lasted for 30 s and during the final two seconds the US was presented. Immediate freezing was scored during the 120 s inter-trial interval. Thirty seconds following the second CS-US pairing the house light was extinguished and mice were returned to their home cages. To test for contextual fear conditioning mice were placed in the training apparatus 24 hours following training and observed for freezing for 5 minutes. At the beginning of the testing session the house light was activated. During testing no CS was presented. Cued fear conditioning was not assessed because multiple studies have shown that withdrawal from chronic nicotine does not alter cued fear conditioning (Davis et al., 2005; Davis and Gould, 2007; Davis et al., 2007; Portugal and Gould, 2007; Portugal et al., 2008).

Drug Administration

Varenicline, generously provided by Pfizer (New York, NY), was dissolved in saline and administered subcutaneously one hour before training and/or testing for all experiments. During experiments in which varenicline was administered at both training and testing, mice were treated with 0, 0.01, 0.1, or 1 mg/kg varenicline; only the 0.1 mg/kg dose or saline was used in experiments where varenicline was administered only at training or at testing. Doses and timing of drug administration were based on communications with Dr. Hans Rolema, of Pfizer.

For experiments involving nicotine withdrawal, nicotine hydrogen tartrate salt (Sigma, St. Louis, MO) was dissolved in saline and administered via mini-osmotic pumps (model 1002; Alzet, Cupertino, CA) at a dose of 6.3 mg/kg/day (dose reported in freebase). The selection of this dose of chronic nicotine was based on previous studies that reported nicotine withdrawal deficits in contextual fear conditioning (Davis et al., 2005; Davis and Gould, 2007; Portugal and Gould, 2007; Portugal et al., 2008) and research demonstrating that this dose of chronic nicotine produces plasma nicotine levels within the range observed in smokers (Benowitz et al., 1989; Henningfield and Keenan, 1993; Davis et al., 2005). On day 1, mini-osmotic pumps were implanted subcutaneously into anesthetized mice. Chronic nicotine or saline was administered for 12 days following pump implantation. All pumps were removed on day 12. Training and testing took place on days 13 and 14, respectively.

Experimental Design

The first experiment examined if varenicline at training and testing in control and nicotine withdrawn mice altered fear conditioning. Because the middle dose of varenicline ameliorated nicotine withdrawal deficits in learning, a second series of experiments were conducted to test

whether varenicline alters processes involved in acquisition or recall in nicotine-withdrawn mice. Thus, for one condition, control mice and nicotine-withdrawn mice received varenicline or saline at training and for the other condition control and withdrawn mice received varenicline or saline at testing.

Statistical Analyses

Data were analyzed with one-way ANOVAs. Homogeneity of variance was tested with the Levene statistic (Cohen, 2001). Tukey post hoc tests were conducted on data sets meeting the homogeneity of variance assumption (Cohen, 2001). Games-Howell post hoc tests were conducted on data sets not meeting the homogeneity of variance assumption (Maxwell & Delaney, 2003). Differences between groups exceeding ($p < 0.05$) are reported as significantly different.

Results

The effects of varenicline on contextual conditioning

To determine if varenicline altered contextual conditioning, varenicline (0, 0.01, 0.1, or 1 mg/kg) was administered to drug-naïve mice prior to training and testing of contextual fear conditioning. Varenicline had no significant effect on measures of baseline, immediate, or contextual freezing (Figure 1A), suggesting that varenicline has no effect on contextual conditioning at these doses.

The effects of varenicline on nicotine withdrawal-induced deficits in contextual conditioning

To determine the effect of varenicline on nicotine withdrawal-induced deficits in contextual conditioning, varenicline (0, 0.01, 0.1 & 1 mg/kg) was administered to nicotine-withdrawn mice prior to the training and testing of contextual fear conditioning. An ANOVA revealed significant differences in contextual freezing between drug treatment groups [$F(4, 34) = 10.72$, $p < 0.05$], but showed no effect of drug condition on either baseline or immediate freezing. Tukey's post-hoc tests demonstrated that nicotine-withdrawn mice receiving 0, 0.01, or 1 mg/kg varenicline froze significantly less than saline-withdrawn mice, and that nicotine-withdrawn mice receiving 0.1 mg/kg varenicline were significantly different from nicotine-withdrawn mice receiving 0, 0.01, or 1 mg/kg varenicline and not significantly different from mice withdrawn from saline (Figure 1B). These results suggest that varenicline dose-dependently ameliorates nicotine withdrawal-induced deficits in contextual conditioning.

The effects of varenicline on the acquisition or recall of contextual fear conditioning

As we were interested in determining whether the effects of varenicline on nicotine withdrawal-induced deficits in contextual conditioning occur during training or testing, we first administered varenicline (0.1 mg/kg) to drug-naïve mice prior to either training or testing of contextual conditioning to determine if varenicline had any effect if administered at those time points. Varenicline, administered at either training or testing, did not significantly alter baseline, immediate, or contextual freezing (Figure 2A), suggesting that varenicline (0.1 mg/kg) has no effect on contextual conditioning if administered only at training or testing.

The effect of varenicline on acquisition or recall during withdrawal from chronic nicotine

To determine if varenicline ameliorates nicotine withdrawal-induced deficits in contextual conditioning by affecting processes that occur during training or testing, we administered varenicline (0.1 mg/kg) to nicotine-withdrawn mice prior to either training or testing of contextual conditioning. An ANOVA revealed significant differences in contextual freezing between drug treatment groups [$F(3,33) = 6.08$, $p < 0.05$], but no differences in baseline or immediate freezing. Games-Howell post hoc tests showed that nicotine-withdrawn mice froze

significantly less than saline-withdrawn controls, that varenicline administered at training to nicotine-withdrawn mice resulted in freezing that was no different than saline-withdrawn controls, and that varenicline treatment at testing of nicotine-withdrawn mice resulted in an intermediate level of freezing which was not significantly different from saline- or nicotine-withdrawn mice (Figure 2B). These results suggest that varenicline ameliorates nicotine withdrawal-induced deficits in processes related to the training of contextual conditioning.

Discussion

This study is the first to demonstrate that varenicline ameliorates nicotine withdrawal-induced deficits in cognitive processing. Specifically, varenicline reversed withdrawal-induced deficits in contextual conditioning in C57BL/6 mice. These findings suggest that varenicline may be effective in ameliorating the nicotine-withdrawal associated cognitive deficits that are reported in human smokers (Bell et al, 1999; Blake & Smith, 1997; Hughes et al, 1989; Jacobsen et al, 2005; Mendrek et al, 2006; Rukstalis et al, 2005). In addition, varenicline administered only at training is sufficient to ameliorate nicotine withdrawal-induced deficits in contextual conditioning, suggesting that varenicline may ameliorate nicotine withdrawal-induced deficits in processes related to the acquisition or consolidation of contextual learning. Although varenicline administration at testing did not reverse nicotine withdrawal-induced deficits in contextual conditioning, an intermediate effect was observed (Figure 2B). This suggests that varenicline may also act to ameliorate nicotine withdrawal-induced deficits in retrieval of contextual learning.

The mechanisms underlying varenicline's amelioration of nicotine withdrawal-induced deficits in contextual conditioning have yet to be determined. Varenicline is a partial agonist for α4β2 nAChRs (i.e. high-affinity nAChRs) (Mihalak et al., 2006) and the high-affinity nAChR antagonist DHβE precipitates deficits in chronic nicotine treated mice. Further, nicotine withdrawal-induced deficits in contextual conditioning do not occur in β2 knockout mice (Portugal et al, 2008). Thus, it seems likely that varenicline ameliorates nicotine withdrawal-induced deficits in contextual conditioning through its action on the α4β2 nAChR. However even though α7 nAChRs are not critically involved in contextual fear conditioning (Davis & Gould; 2007; Wehner et al, 2004), it is possible that varenicline also acts on the α7 nAChR to ameliorate nicotine withdrawal-induced deficits in this task. Further research is necessary to determine which of these nicotinic receptors is involved in the effects of varenicline on nicotine withdrawal-induced deficits in contextual conditioning.

This study adds to previous research suggesting that contextual fear conditioning could be a useful screening tool for potential smoking cessation agents. First, deficits in contextual fear conditioning can be induced by withdrawal of a dose of chronic nicotine that produces plasma nicotine levels within the range reported in human smokers (Benowitz et al, 1982; Benowitz, 1988; Davis et al, 2005). Additionally, withdrawal-induced deficits in contextual fear conditioning can be ameliorated by smoking cessation therapies including; acute nicotine (NRT), bupropion, and varenicline (Davis et al, 2005; Portugal & Gould, 2007). Another strength of this animal model is that the effects of nicotine withdrawal on learning observed in our model are consistent with the disruption of cognition that is reported during human smoking cessation (Hughes et al, 1991; Jacobsen et al, 2005; Mendrek et al, 2006, Hughes, 2007; Jacobsen et al, 2007). In contrast, other models have focused on somatic withdrawal symptoms (Malin et al, 1992; Malin, 2001), which may not reflect the typical symptoms reported during nicotine withdrawal in humans (West and Gossop, 1004; Hughes, 2007). Therefore, an animal model of cognitive withdrawal symptoms may identify different neural substrates of nicotine withdrawal and potential therapeutic targets than those indicated by a model of somatic withdrawal signs.

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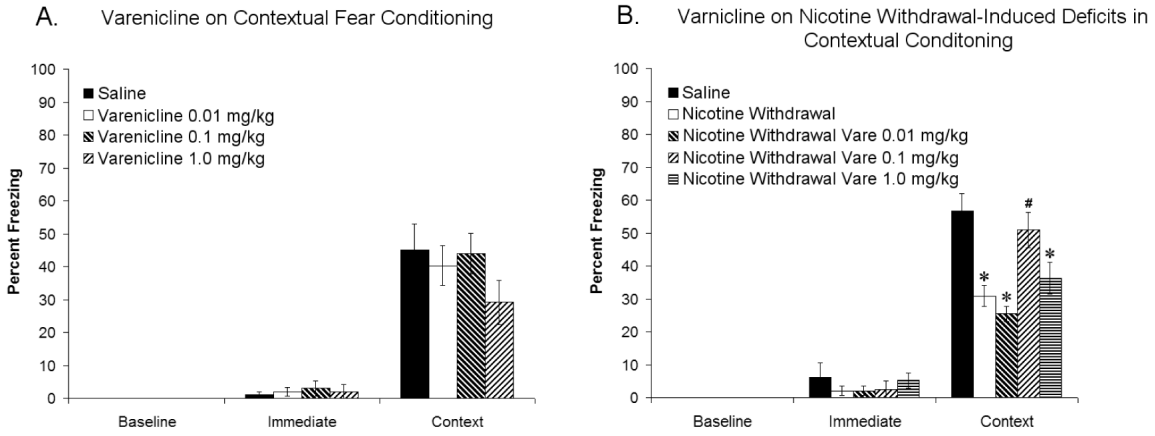

Figure 1.

Varenicline at doses of 0.01, 0.1, and 1.0 mg/kg does not affect contextual fear conditioning (Figure 1A). Deficits in contextual fear conditioning produced by withdrawal of chronic nicotine administration are ameliorated by varenicline (0.1 mg/kg) (Figure 1B). Significant difference ($p < 0.05$) from saline treated groups denoted with (*), significant difference ($p < 0.05$) from nicotine withdrawal groups denoted by (#).

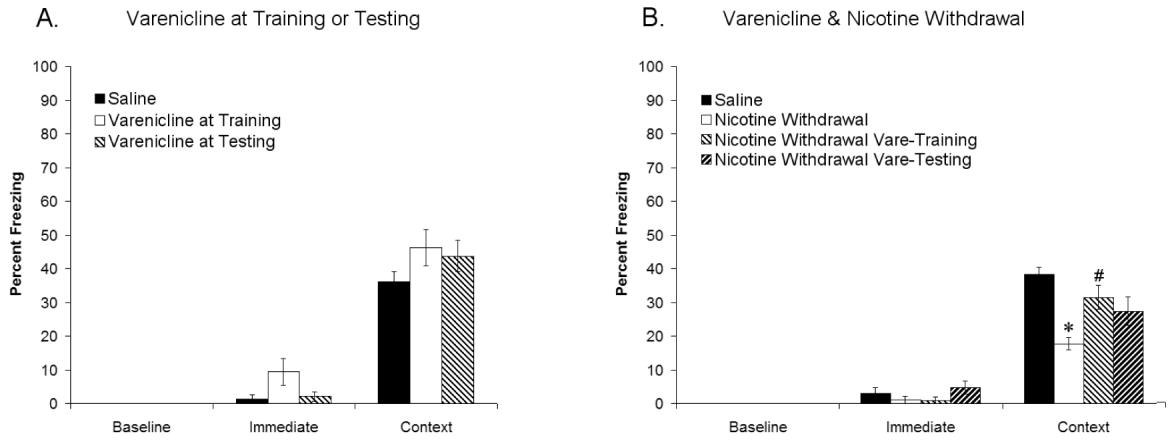

Figure 2.

Varenicline (0.1 mg/kg) has no effect on contextual fear conditioning if administered only at training or testing (Figure 2A). Deficits in contextual fear conditioning produced by withdrawal of chronic nicotine administration are ameliorated by varenicline at training (Figure 2B). Significant difference ($p < 0.05$) from saline treated groups denoted with (*), significant difference ($p < 0.05$) from nicotine withdrawal groups denoted by (#).