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The effects of galantamine on nicotine withdrawal-induced deficits in contextual fear conditioning in C57BL/6 mice

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Abstract

Current smoking cessation are relatively ineffective at maintaining abstinence during withdrawal. Nicotine withdrawal is associated with a variety of symptoms including cognitive deficits and targeting these deficits may be a useful strategy for maintaining abstinence. Galantamine is an acetylcholinesterase inhibitor and allosteric modulator of nicotinic acetylcholine receptors (nAChRs) with cognitive enhancing effects that may alleviate cognitive deficits associated with nicotine withdrawal. The effects of galantamine on nicotine withdrawal-induced deficits in contextual fear conditioning in C57BL/6 mice were examined. An initial acute dose-response experiment revealed that 0.5 and 1 mg/kg galantamine had no effect on fear conditioning. To determine if galantamine would reverse nicotine withdrawal-related deficits in contextual fear conditioning, mice were implanted with osmotic mini-pumps that delivered chronic saline or 6.3 mg/kg/d nicotine for 12 days and then pumps were removed. Training and testing of fear conditioning occurred 24 and 48 hours later, respectively. Nicotine withdrawal disrupted contextual fear conditioning, which was reversed with 1 but not 0.5 mg/kg galantamine. Across all conditions in both studies 2 mg/kg galantamine led to high levels of freezing that were likely due to nonspecific effects. The ability of galantamine to reverse nicotine withdrawal-deficits in contextual conditioning is likely mediated through enhanced levels of acetylcholine via inhibition of acetylcholinesterase, potentiation of hippocampal $\alpha 4\beta 2^*$ nAChRs, or both. The present study suggests that acetylcholinesterase inhibitors and/or drugs that act as allosteric modulators of nAChRs might be targets for smoking cessation aids because they may alleviate withdrawal symptoms such as cognitive deficits that can lead to relapse.

Keywords

Nicotine; withdrawal; learning and memory; acetylcholine; addiction; cognition

1. Introduction

Cigarette smoking is a serious health concern in the United States - with approximately one in five adults smoking despite known health risks including approximately 443,000 deaths per year [1]. The continued use of tobacco products in the face of clear health risk is because nicotine, the main psychoactive substance in tobacco products, is a highly addictive drug.

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The addictive strength of this drug is best illustrated by the fact that over 70% of smokers wish to quit, 40% make an attempt every year, and yet only 3 to 5% of these smokers are successful [2]. For those who wish to quit smoking, there are three available FDA approved treatments: varenicline, bupropion, and nicotine replacement therapies such as nicotine gum [3]. Unfortunately, only one out of five smokers are able to maintain abstinence with these aids [4]. The low rates of success in quitting for smokers may be due, in part, to the efficacy of currently available smoking cessation aids at maintaining abstinence during withdrawal.

When smokers try to quit, they may experience a variety of withdrawal symptoms that include cravings, cognitive deficits, irritability, and a depressed mood [5–9]. The cognitive deficits include reduced sustained attention [10, 11], impaired working memory [11, 12], and visuospatial memory deficits [13]. Moreover, one study found that 66% of smokers reported difficulty concentrating during a nicotine withdrawal period [14]. Avoidance or alleviation of these symptoms may contribute to continued nicotine use and/or relapse upon cessation [5, 11, 15, 16]. In fact, changes in cognition associated with nicotine withdrawal are able to predict relapse after brief periods of smoking cessation [10]. The cognitive deficits from nicotine withdrawal can be reversed with nicotine [15] as well as nicotine replacement therapies [17], bupropion [18], and varenicline [19]. While these treatments have varying degrees of success, developing more effective treatments for nicotine withdrawal-deficits in cognition may facilitate abstinence. Acetylcholine is an important neurotransmitter for learning and memory [20, 21] and is the target system for nicotinic drugs. Single nucleotide polymorphisms in the gene encoding choline acetyltransferase (*ChAT*), an enzyme responsible for the synthesis acetylcholine, have been linked to nicotine dependence and success in smoking cessation, although the functional significance of these polymorphisms is unknown [22]. Thus, cholinergic drugs with known cognitive enhancing effects may be useful in preventing relapse due to cognitive deficits during periods of abstinence.

Galantamine is a cognitive enhancing cholinergic drug that inhibits acetylcholinesterase, the enzyme that degrades acetylcholine, and also acts as an allosteric positive modulator of nicotinic acetylcholine receptors (nAChRs), potentiating nAChR response to acetylcholine and other nicotinic agonists [23–26]. In addition, this drug alleviates some of the cognitive deficits [27, 28] related to Alzheimer's disease. Thus, galantamine may be useful in alleviating cognitive deficits associated with nicotine withdrawal. The following experiments examined the effects of galantamine treatment on nicotine withdrawal-induced deficits in contextual fear conditioning.

2. Material and methods

2.1. Subjects

Subjects were male C57BL/6 mice aged 8–12 weeks at training (N = 115) (Jackson Laboratories, Bar Harbor, ME). Mice were housed four per cage and were provided *ad libitum* access to food and water. A 12 hr light-dark cycle was maintained throughout the study and all experimental procedures were conducted during the light cycle. The Temple University Institutional Animal Care and Use Committee approved all procedures.

2.2. Surgeries

For all experiments, mice were anesthetized with isoflurane (2–5%) and surgically implanted with subcutaneous osmotic mini-pumps (Alzet, Model 1002, Durect Co, Cupertino, CA) that delivered saline or 6.3 mg/kg/d nicotine s.c. for 12 days. In the acute dose-response experiment, mice were implanted with osmotic mini-pumps that delivered

saline only. A second surgery was performed 12 days after implantation in which the osmotic mini-pumps were removed to induce saline or nicotine withdrawal.

2.3. Drugs and Administration

Nicotine hydrogen tartrate salt (Sigma, St. Louis, MO) and galantamine hydrobromide (Tocris Bioscience, Ellisville, MO) were dissolved in 0.9% saline. Galantamine (0.5, 1, and 2 mg/kg, doses based on previous research [29, 30]) or saline was administered i.p. 20 minutes prior to training and testing of fear conditioning. Nicotine (6.3 mg/kg/d, dose reported in freebase weight) or saline was administered s.c. via osmotic-mini pump for 12 days; dose based on [8].

2.4. Apparatus

Mice were trained and tested for contextual conditioning in four identical clear Plexiglas chambers (26.5 × 20.4 × 20.8 cm) housed in sound attenuating boxes (Med-Associates, St. Albans, VT). The floor of each chamber was made of metal bars connected to a shock generator and scrambler (Med Associates, Model ENV-414). Ventilation fans were mounted on the sides of each box to provide background noise. A 4 W light was mounted above each box for illumination. White noise and shock administration was controlled by a PC running LabView software. Testing for cued conditioning occurred in an altered context consisting of four chambers (20.3 × 22.9 × 17.8 cm) housed in sound attenuating boxes (Med-Associates) in a different room from the training room. The floor of each chamber was made of white plastic. Speakers were mounted on the left wall of each chamber to deliver the CS. Vanilla extract was added to a tray beneath the floors to further distinguish the chambers from the training chambers. All chambers were cleaned with 70% ethanol before and after all behavioral procedures.

2.5. Behavioral Procedure

The behavioral procedure was performed as previously reported [31]. Freezing, defined as the complete absence of movement besides respiration, was sampled live for 1 s every 10 s and was used as a measure of learning and memory. The experimenter was trained to 95% interobserver reliability with an experienced observer prior to the initiation of all behavioral procedures. On training day, mice were transported from the colony room to a holding room on the same floor as the training and testing chambers and remained in the holding room for 1 hour before behavioral procedures began. Mice were then placed into one of the four chambers and then baseline freezing was scored for 120 s. This was followed by two auditory cue CS (30 s 85 dB white noise)-US (2 s 0.57 mA footshock) pairings separated by a 120 s ITI. After the last CS-US pairing, mice remained in the chambers for an additional 30 s before being returned to their homecages in the holding room. Mice sat in the holding room for 1 hour before being returned to the colony room.

The next day, mice were transported to the holding room and remained for 1 hour before testing began. Mice were then placed back into the original training context without the CS for 5 min and freezing to the context freezing was scored for 5 min. Mice were then returned to their homecages in the holding room. Approximately one hour later, mice were placed in the altered context for a total of 6 min. Generalized freezing was scored for the first 3 min in the absence of the CS. The CS was then turned on and cued freezing was scored for 3 min. The experimenter was blind to all experimental conditions.

2.6. Statistical Analyses

Data were analyzed using one-way ANOVA's, followed by Tukey's Honestly Significant Difference tests. Statistical significance was set at the $p < 0.05$ level.

3. Results

3.1. Effects of acute galantamine on fear conditioning

To determine if acute galantamine affected fear conditioning, mice were trained and tested in fear conditioning after acute injections of galantamine (Figure 1). All mice in this acute experiment underwent withdrawal from chronic saline (WCS) to allow for a better comparison to the nicotine withdrawal study. Galantamine (0.5, 1, and 2 mg/kg) or saline ($n = 10$) was administered prior to training and testing of fear conditioning. A one-way ANOVA revealed a significant effect of galantamine treatment on contextual ($F(3, 36) = 13.768$, $p < 0.01$), baseline ($F(3, 36) = 15.783$, $p < 0.01$), and pre-CS ($F(3, 36) = 21.591$, $p < 0.01$) freezing. Freezing to the cue was not affected by galantamine treatment ($F(3, 36) = 1.119$, $p > 0.05$). Post-hoc tests revealed that mice administered 2 mg/kg ($n = 10$) galantamine froze significantly more than all other groups during baseline, contextual, and pre-CS (all p 's < 0.01). The high levels of freezing from 2 mg/kg galantamine across all conditions indicates this dose may not specifically enhance learning and memory but affects other systems, such as those involved in locomotion, that also lead to high levels of freezing. Together, these results demonstrate that 0.5 ($n = 10$) and 1 mg/kg ($n = 10$) did not affect contextual or cued fear conditioning.

3.2. Effects of acute galantamine on nicotine withdrawal-induced deficits in contextual conditioning

Next, to determine if acute galantamine treatment would reverse nicotine withdrawal-induced deficits in contextual conditioning, mice undergoing WCS or withdrawal from chronic nicotine (WCN) were trained and tested in fear conditioning after acute injections of galantamine (0.5, 1, and 2 mg/kg) or saline (Figure 2). A one-way ANOVA revealed a significant effect on contextual ($F(4, 70) = 13.143$, $p < 0.01$), baseline ($F(4, 70) = 33.669$, $p < 0.01$), and pre-CS ($F(4, 70) = 17.392$, $p < 0.01$) freezing. Cued freezing was not affected by galantamine treatment ($F(4, 70) = 2.214$, $p > 0.05$). Post-hoc tests revealed that WCN mice administered saline ($n = 15$) and WCN mice administered 0.5 mg/kg ($n = 15$) galantamine froze significantly less to the context than all other groups (all p 's < 0.05). There was no significant difference in contextual freezing between WCS mice administered acute saline ($n = 15$) and WCN mice administered 1 ($n = 15$) or 2 mg/kg ($n = 15$) galantamine (p 's > 0.05). However, the high levels of freezing (in both baseline and pre-CS conditions) for the 2 mg/kg galantamine group may indicate that associated effects from the 2 mg/kg dose result from something other than or in addition to changes in learning and memory. Thus, galantamine (1 mg/kg) reversed nicotine withdrawal-related deficits in contextual conditioning.

4. Discussion

The present study sought to determine the efficacy of galantamine in reversing cognitive deficits induced by withdrawal from chronic nicotine. Nicotine withdrawal impaired contextual conditioning consistent with previous research [15, 18, 19, 32–36]. During nicotine withdrawal, 0.5 mg/kg galantamine did not alter contextual conditioning in saline- or nicotine-withdrawn mice; however, 1 mg/kg galantamine reversed nicotine withdrawal-induced impairments in contextual conditioning. Interestingly, 1 mg/kg galantamine did not affect contextual conditioning administered to mice withdrawn from chronic saline but was able to reverse impairments in contextual conditioning produced by nicotine withdrawal. The enhanced levels of freezing from 2 mg/kg galantamine across all conditions in both experiments are most likely due to nonspecific effects such as a reduction of locomotor activity. Prior research has demonstrated that the effective dose of galantamine in the present study (1 mg/kg) does not significantly affect locomotor activity in mice [37] while

higher doses (such as 2.5 and 10 mg/kg galantamine) reduce locomotion [38]. These results are consistent with the interpretation of the present results whereby galantamine had dose-dependent effects on locomotor activity but the 1 mg/kg dose did not change cued or contextual freezing in chronic saline treated mice. Thus, the 1 mg/kg dose of galantamine appears to be effective in ameliorating nicotine withdrawal-associated deficits in contextual fear conditioning in mice. Taken together, the results of the present study suggest that galantamine may serve as a useful pharmacotherapy for treating the cognitive deficits associated with nicotine withdrawal.

There are at least two possible mechanisms, operating alone or in combination, through which galantamine may reverse cognitive deficits from nicotine withdrawal. First, galantamine enhances cholinergic transmission by inhibiting acetylcholinesterase [23]. The importance of acetylcholine in cognition has been well documented [20, 21] and changes in cholinergic activity often correspond to changes in cognitive function [27, 39]. For example, microdialysis studies have shown an increase in extracellular levels of acetylcholine during many cognitive functions including attention [40], exposure to novelty [41], working memory [42], and spatial memory [43]. Pharmacological inhibition of acetylcholine and lesions of the cholinergic system often produce deficits in learning and memory (see [32] for a review). Alterations in endogenous cholinergic transmission is postulated to be one biochemical mechanism underlying nicotine withdrawal [6] and therefore galantamine might reverse nicotine withdrawal cognitive deficits by restoring cholinergic activity to pre-withdrawal levels.

Second, galantamine acts as an allosteric potentiating ligand at nAChRs [24, 25, 44]. Allosteric potentiating ligands of nAChRs bind to sites that are distinct from those of acetylcholine and nicotinic agonists and potentiation or enhance nAChR responsivity to acetylcholine and other nicotinic agonists [24, 25, 44, 45]. The binding sites for nicotinic agonists on nAChRs lie at the interface of α and β subunits except in the case of homomeric receptors where binding sites are formed at the interface between α subunits [46–48]. Galantamine on the other hand binds to α subunits of nAChRs allowing it to modulate nAChR responsivity to nicotinic agonists without interfering with agonist binding [44]. Hippocampal nAChRs containing $\beta 2$ subunits, possibly $\alpha 4\beta 2^*$ nAChRs (the * indicates other subunits may be incorporated into this receptor) mediate nicotine withdrawal-deficits in contextual conditioning [34, 35, 49] and agonists of this receptor such as nicotine [15] and the partial agonist varenicline [19] are able to reverse these deficits. Evidence indicates that galantamine binds to $\alpha 4\beta 2^*$ nAChRs [25, 50, 51] and therefore may enhance hippocampal $\alpha 4\beta 2^*$ nAChR responsivity to endogenous acetylcholine. This would compensate for reduced cholinergic function during nicotine withdrawal and therefore lead to an alleviation of the cognitive withdrawal symptoms.

The ability of galantamine to reverse cognitive deficits in the present study is in agreement with multiple studies showing galantamine improves cognition. Galantamine reduces scopolamine induced deficits in passive avoidance and enhances learning in step-down passive avoidance [52]. Likewise, galantamine facilitates trace eyeblink conditioning in both aged and young rabbits [53, 54]. Lesions of the nucleus basalis magnocellularis, which provides cholinergic input to the frontal-parietal cortex, produces deficits in Morris water maze performance that are reversed by galantamine treatment [30, 55]. Many studies designed to assess the pro-cognitive effects of galantamine have examined the ability of galantamine to ameliorate cognitive deficits caused by experimental manipulation or normal aging [29, 30, 50, 52, 55–61]. However, when galantamine is administered alone or to control animals it often impairs or produces no change in cognition [26, 29, 30, 50, 55, 57, 59]. Consistent with this, the present study demonstrated that 1 mg/kg galantamine did not affect contextual conditioning alone but was able to reverse cognitive deficits produced by

nicotine withdrawal. This may be ideal from a clinical standpoint because galantamine could restore cognitive function to normal levels when there is a pre-existing cognitive impairment (e.g., nicotine withdrawal) without altering normal cognitive function.

Unfortunately, a search of the literature and recent review articles reveals that there is a lack of clinical studies that have examined cognitive improvement in healthy human smokers or nonsmokers following treatment with galantamine [27, 62]. In addition, there are relatively few studies on the effects of galantamine on smoking in any population. In patients with schizophrenia, galantamine does not improve cognitive function in nonsmokers or smokers [63–66] and does not reduce cigarette smoking in smokers [66]. In fact, galantamine slightly increases ratings of nicotine dependency and cravings [66]. While the study by Kelly et al. [59] indicates that galantamine may actually worsen smoking cessation rates in populations with schizophrenia, galantamine has been shown to be effective in reducing smoking in people with alcohol dependency [67]. The efficacy of galantamine in preventing relapse to smoking during abstinence may be dependent upon factors such as mental illness, comorbid drug dependency, and possibly genetic variability.

5. Conclusion

In conclusion, galantamine is able to reduce nicotine withdrawal-deficits in contextual conditioning. This may be due to galantamine potentiating $\alpha 4\beta 2^*$ nAChR responsiveness to endogenous acetylcholine, enhancing cholinergic transmission, or both. The ability of galantamine to improve cognitive function following nicotine withdrawal may depend upon preexisting conditions. Future studies could evaluate the efficacy of acetylcholinesterase inhibitors and/or allosteric modulators of nAChRs as a pharmacotherapy for maintaining smoking abstinence and preventing relapse in healthy human subjects.

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Figure 1.

The effects of acute galantamine on fear conditioning ($n = 10$ for each dose group). WCS = withdrawal from chronic saline. * = significantly different from WCS + Saline. Error bars represent $\pm$ SEM. Freezing to the training context (baseline) was assessed in the absence of the auditory cue and footshock. Contextual fear conditioning (context) was measured by placing the animals back in the training context a day after training. Cued fear conditioning was measured by placing the animals in the altered context for 3 minutes without the tone (pre-CS) then freezing to the tone was measured (CS) for 3 minutes.

Figure 2.

The effects of acute galantamine on nicotine withdrawal induced deficits in contextual conditioning ($n = 15$ for each dose group). WCS = withdrawal from chronic saline. WCN = withdrawal from chronic nicotine. * = significantly different from WCS + Saline. Error bars represent $\pm$ SEM. Freezing to the training context (baseline) was assessed in the absence of the auditory cue and footshock. Contextual fear conditioning (context) was measured by placing the animals back in the training context a day after training. Cued fear conditioning was measured by placing the animals in the altered context for 3 minutes without the tone (pre-CS) then freezing to the tone was measured (CS) for 3 minutes.