Published in final edited form as:

Alcohol Clin Exp Res. 2010 December ; 34(12): 2053–2060. doi:10.1111/j.1530-0277.2010.01301.x.

The nicotinic acetylcholine receptor partial agonist varenicline increases the ataxic and sedative-hypnotic effects of acute ethanol administration in C57BL/6J mice

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Abstract

Background—The costs associated with alcohol abuse are staggering, therefore much effort has been put into developing new pharmacological strategies to decrease alcohol abuse. Recently, the nicotinic acetylcholine receptor (nAChR) partial agonist varenicline has been shown to decrease ethanol consumption in both humans and animal models.

Methods—We examined the effects of varenicline on the ataxic and sedative-hypnotic effects of ethanol. First, varenicline was administered prior to placement in a locomotor activity chamber to determine if varenicline influenced baseline locomotor activity. To determine the effect of nicotinic modulation on ethanol-induced motor incoordination, varenicline was administered 30 min prior to an acute ethanol injection and then mice were tested on the balance beam, dowel test or fixed-speed rotarod. To examine ethanol's sedative-hypnotic effects, varenicline was administered 30 min prior to 4 g/kg ethanol and the duration of loss of righting reflex (LORR) was measured.

Results—Varenicline markedly reduced baseline locomotor activity in C57BL/6J mice. Varenicline increased ethanol-induced ataxia when measured on the balance beam and dowel test, but had no effect when measured on the fixed-speed rotarod. Pretreatment with varenicline increased the duration of LORR.

Conclusions—These data provide evidence that nAChRs may be involved in the ataxic and sedative effects of ethanol. It is possible that one mechanism which could contribute to the ability of varenicline to decrease ethanol consumption may be through increasing negative behavioral effects of alcohol.

Keywords

Ethanol; Varenicline; Nicotinic Acetylcholine Receptors; Ataxia; Sedation

INTRODUCTION

There is a substantial social and economic burden that can be attributed to alcohol consumption. The excessive use of alcohol can increase the risk of a number of health problems, including: cancer (Baan *et al.*, 2007), cardiovascular problems (Rehm *et al.*,

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DISCLOSURE/CONFLICTS OF INTEREST

In 2007, MRP received a small grant from Pfizer to study the effects of varenicline in animal models of depression. The authors have no current financial conflicts of interest to disclose.

2003), liver disease (Schiff, 1997) and neurological problems (Corrao et al., 2004; Corrao et al., 2002). The cost associated with alcohol consumption is a staggering \$185 billion per year (Harwood, 1998). Together, these data provide a strong rationale for the continued effort to develop effective pharmacotherapies for alcohol abuse.

Alcohol and nicotine are commonly co-abused (Madden et al., 2000; Talcott et al., 1998) and there is considerable evidence that nicotinic acetylcholine receptors (nAChRs) play a role in the behavioral and physiological effects of ethanol (for review see Dani and Harris, 2005; Larsson and Engel, 2004; Li et al., 2007). Given the accumulating genetic data suggesting that there are common genes underlying responses to alcohol and nicotine (Bergstrom et al., 2003; Collins et al., 1993; de Fiebre et al., 1991; de Fiebre et al., 2002; Kendler et al., 2007; Madden and Heath, 2002), drugs that alter nAChR function have become of interest as potential treatments for alcohol abuse disorder. In 2006, the FDA approved varenicline (Chantix®), an $\alpha 4\beta 2$ nAChR partial agonist, as a smoking cessation aid (Coe et al., 2005; Reus et al., 2007; Rollema et al., 2007a; Rollema et al., 2007b) and this drug has been quite successful in decreasing smoking and relatively well tolerated (Cahill et al., 2008).

A number of studies have implicated varenicline as a potential pharmacotherapy for alcohol abuse and alcoholism. Preclinical studies in mice and rats (Kamens et al., 2010; Steensland et al., 2007) and a clinical study in heavy drinking smokers (McKee et al., 2009) have provided evidence that varenicline can decrease ethanol intake. Furthermore, varenicline blocks the increase in extracellular dopamine in the nucleus accumbens observed in rats following an acute ethanol injection (Ericson et al., 2009). These data provide evidence that varenicline might decrease the rewarding properties of ethanol. In addition to decreasing the rewarding effects, it is possible that varenicline might decrease ethanol consumption due to an increase in ethanol's adverse behavioral effects. To date, the effect of varenicline on other ethanol-related behaviors has not been tested.

The function of nAChRs has been implicated previously in ethanol's ataxic and sedative-hypnotic effects. In a recent paper, the $\alpha 4\beta 2$ selective agonist RJR-2403 was injected into the cerebellum and decreased ethanol-induced ataxia (Taslim et al., 2008). These data support the hypothesis that nAChRs, in particular those expressed in the cerebellum, are involved in the ataxic effects of ethanol. Furthermore, mice lacking the $\alpha 7$ nAChR subunit have a longer duration of loss of righting reflex (LORR, a common measure of ethanol's sedative properties) compared to wild-type mice (Bowers et al., 2005). In the current set of experiments we set out to determine the effects of varenicline on ethanol-induced behaviors. Since prior work has shown that varenicline decreases ethanol consumption, we wanted to explore if varenicline also affects the ataxic and sedative-hypnotic effects of ethanol. We used 3 behavioral measures of ethanol-induced ataxia: balance beam, dowel test, and fixed-speed rotarod. To measure the sedative-hypnotic effects of ethanol we tested the duration of LORR. We hypothesized that varenicline might alter acute ethanol-induced ataxia and sedation as it has already been shown to alter ethanol choice following chronic ethanol drinking.

MATERIALS AND METHODS

Male and female C57BL/6J mice purchased from The Jackson Laboratory (Bar Harbor, ME) were used in all experiments. All mice were housed 2-4 per cage in standard mouse cages. Water and rodent chow (Harlan Teklad #2018) was available ad libitum. The colony room was maintained on a 12-h light/dark cycle (lights on a 0700) at $21 \pm 2^\circ\text{C}$. Behavioral testing took place between 0900-1700h. Mice were 11-12 weeks of age at the start of testing. All studies were approved by the Yale Animal Care and Use Committee.

Drugs

All drugs were prepared fresh on the day of testing in Dulbecco's Phosphate Buffered Saline (saline; Invitrogen, Grand Island, NY). Two-hundred proof ethanol was purchased from Pharmco (Brookfield, CT) and was diluted to a 20% v/v solution for injections. Varenicline tartrate was generously provided by Pfizer (Groton, CT) and was injected i.p. at a volume of 10 ml/kg. All doses refer to active varenicline base. Varenicline doses were based on our prior experiments in C57BL/6J mice (Kamens et al., 2010).

Locomotor Activity

Locomotor activity was measured in 47 C57BL/6J mice in eight automated activity monitors (Med Associates, St. Albans, VT). Each apparatus was composed of a 17 × 10 × 9.5-inch (length × width × height) clear acrylic cage placed into an activity monitor. Each monitor had 16 photo beams placed approximately 1 inch apart along the length of the cage. Locomotor activity was defined as the crossing of 2 consecutive beams. Experimentally naïve mice were tested in a 3-day paradigm; groups were assigned prior to testing on Day 1. On Days 1 and 2 mice were given an injection of saline before being placed into the activity monitor for 60 min. This allowed mice to habituate to the injection and testing environment. On Day 3 animals received an injection of saline, 1 or 2 mg/kg varenicline before being placed into the monitoring apparatus. Activity was recorded in 5 min epochs for 60 min.

Balance Beam

The balance beam was used to examine ethanol-induced ataxia using a procedure adapted from what has been reported previously (Crabbe et al., 2003b; Linsenbardt et al., 2009). The balance beam apparatus consisted of a white, 104.1 cm long by 1.9 cm wide, PVC board mounted on supports on both ends. The balance beam was elevated 54.6 cm above the floor. Disposable pads placed under the beam provided cushioning if an animal came off the beam. One hundred twenty-two experimentally naïve mice were trained to traverse the beam at least 1 hr prior to testing. To complete the training, each mouse had to walk across the beam twice. If a mouse stopped during training the tail was gently pressed to encourage movement. Following training mice were left undisturbed for at least an hour prior to testing. Mice were given an injection of saline or varenicline (0.5, 1, or 2 mg/kg) and placed into individual holding cages for 30 min. This pretreatment time was consistent with our prior work that examined the effect of varenicline on ethanol consumption in C57BL/6J mice (Kamens et al., 2010). Mice were then challenged with saline or ethanol (1.5 g/kg); this dose of ethanol was chosen based on prior literature (Linsenbardt et al., 2009) and pilot testing in our laboratory. Ten min after the ethanol challenge each mouse traversed the balance beam and the number of hind foot missteps was counted by an experimenter unaware of the treatment conditions. A hind foot misstep was characterized by the entire paw missing the beam. Similar to training trials, if an animal stopped, its tail was gently pressed to encourage movement. If an animal fell from the beam it was replaced at approximately the same location and allowed to finish traversing the beam (Crabbe et al., 2003b).

Dowel

One week after mice were tested on the balance beam they were tested for ataxia using the dowel test (Crabbe et al., 2003a). Mice were pseudorandomly reassigned to drug conditions so that groups were balanced for previous testing. The dowel was constructed from a 15.9 mm diameter PVC pipe elevated 54.6 cm above the floor. Sand paper was used to roughen the surface of the dowel. The dowel was divided into four 15 cm long sections separated for testing of individual mice. At least 1 h prior to testing mice were trained on the dowel and had to stay on the dowel for at least 2 min to be included in the study. All mice were able to

stay on the dowel for this time. Mice received a pre-injection of saline or varenicline (0.5, 1, or 2 mg/kg) 30 min prior to a saline or ethanol (1.5 g/kg) challenge. Immediately after the challenge injection, mice were placed on the dowel and the latency to fall was recorded with a maximum duration of 300 s. This was repeated 30 min later.

Fixed-Speed Rotarod

Two hundred C57BL/6J mice were tested for motor incoordination using a standard mouse rotarod (ENV-576M; Med Associates Inc, Georgia, VT) based on a published protocol (Taslim et al., 2008). A subset of the mice tested on the rotarod had been tested previously for locomotor activity (at least 2 weeks prior to rotarod testing) and the remainder were experimentally naïve. There were no significant differences in performance between the naïve mice and the ones tested previously for locomotor activity, therefore the groups were combined. Briefly, mice were moved to the procedure room and tested for their ability to remain on the rotarod, rotating at a fixed-speed of 24 rpm. Each animal had up to 5 training trials during which in any one trial they had to remain on the rod for 180 s. Training stopped after an animal reached the criterion once. Animals that did not successfully stay on the rotarod for this time (N=18) were excluded from further testing. Following these training trials, animals were returned to their home cage for at least 30 min prior to testing. Each mouse received a saline or varenicline injection (0, 0.5, 1, or 2 mg/kg) and was placed into a holding cage for 30 min prior to a saline or ethanol (1.5 g/kg) challenge. Immediately after the challenge injection, the mice were tested for their ability to stay on the rotarod (T0). Mice were re-tested every 15 min (T15, T30, T45, and T60). During each trial the latency to fall from the rod was recorded with a maximum duration of 180 s per trial. The area under the curve was used to combine the data from all time points to obtain a single dependent variable (Taslim et al., 2008).

LORR

A subset of mice (N=89) that were tested on the rotarod were left undisturbed in the colony room for 2 weeks before they were tested for sensitivity to the sedative effects of ethanol. Mice were pseudorandomly assigned to new drug conditions so that previous rotarod performance did not affect LORR testing. Sedative effects were measured by the LORR using V-shaped troughs, based on published methods (Crabbe et al., 2006; McClearn and Kitahama, 1981). Briefly, mice were moved to the testing room, weighed and left to habituate for at least 30 min. Mice were injected with varenicline (0, 0.5, 1, or 2 mg/kg) and returned to their home cage for 30 min before being challenged with ethanol (4 g/kg). Following the ethanol injection, each mouse was held gently for approximately 1 min until it appeared to be intoxicated and then was placed on its back in a V-shaped trough. LORR was defined as the animal remaining on its back for at least 30 s. Five mice had not reached LORR within 3 min of ethanol injection. These mice were excluded from further analysis because of a presumed misplaced injection (Ponomarev and Crabbe, 2002). The remaining mice were observed until they righted themselves (defined as turning over onto all four paws) and then was returned to the supine position. A mouse was deemed to have regained its righting reflex when it was able to right itself two times within 30 s.

Statistical Analysis

Primary dependent variables examined were locomotor activity, footslips, latency to fall, and duration of LORR. Data from the locomotor activity, balance beam, and LORR studies were analyzed using factorial analyses of variance (ANOVAs). Significant interactions were subsequently analyzed with simple main effects analyses and Tukey's HSD for post hoc comparisons. Sex, varenicline dose, and epoch were possible independent factors. Data from the rotarod and dowel tests were analyzed using nonparametric statistics since the data were not normally distributed due to a ceiling criterion in each test. For these data, Kruskal-Wallis

tests were performed to determine if varenicline pretreatment had an effect on either saline- or ethanol-induced behavior. Significant effects of pretreatment were followed by Mann-Whitney *U* comparisons. $\alpha < 0.05$ was considered significant.

RESULTS

Locomotor Activity

Acute administration of varenicline reduced baseline locomotor activity in C57BL/6J mice (Fig. 1). Data were originally collected in 5 min epochs, but to simplify the analyses, data were combined into 10 min epochs. Since Days 1 and 2 were used to habituate the animals to the test environment and no significant differences were found between the varenicline groups on Day 2, we focused our analysis on Day 3 when mice were challenged with saline or varenicline. Due to a significant main effect of sex and significant interactions between sex and other variables, data for female and male mice were analyzed independently. In female mice, there was a significant epoch X dose interaction ($F_{10,100}=4.3$, $p<0.001$). Female mice that received 2 mg/kg varenicline displayed significantly less locomotor activity compared to mice that received saline or 1 mg/kg varenicline during the first 40 min of the test. During the 5th epoch of the test, or 50 min after the varenicline injection, there was significantly less activity in mice that had received 2 mg/kg varenicline compared to mice that received 1 mg/kg varenicline, but not saline. In male mice there was also a significant epoch X dose interaction ($F_{10,105}=3.8$, $p<0.001$). Male mice receiving 2 mg/kg varenicline were significantly less active during the first 20 min of the test compared to the male mice that received 1 mg/kg varenicline. During the second epoch there was also a trend ($p=0.06$) for mice receiving 2 mg/kg varenicline to have reduced locomotor activity compared to saline treated mice.

Balance Beam

Varenicline increased ethanol-induced ataxia as measured on the balance beam (Fig. 2). There was no significant main effect of varenicline pretreatment on the number of footslips observed following saline challenge ($p=0.5$); thus to simplify the data we corrected the number of footslips after ethanol by subtracting the footslips made by the respective saline group, a standard way to present these data (Crabbe et al., 2003b). A 2-way ANOVA on the corrected footslips with sex and varenicline dose as independent variables revealed a significant main effect of dose ($F_{3, 58}=2.9$, $p<0.05$), but no other significant effects or interactions. Pretreatment with 2 mg/kg of varenicline increased the number of footslips observed following ethanol administration compared to the saline pretreatment group ($p<0.05$). The 0.5 and 1 mg/kg varenicline groups, neither of which would be expected to affect locomotor activity, also tended ($p=0.1$) to increase footslips compared to saline.

Dowel Test

Varenicline increased ethanol-induced motor incoordination measured in the dowel test in C57BL/6J mice (Fig. 3). Only a few (5 at each time point, but never the same mouse) animals that received a saline challenge were not able to stay on top of the dowel for the full 3 min. Thus to simplify the data we only examined mice that were challenged with an ethanol injection. Immediately after the ethanol injection a significant main effect of pretreatment was observed ($H_{3, N=63} = 8.6$, $p < 0.05$). Mice that received 0.5 mg/kg varenicline prior to ethanol were significantly more ataxic compared to mice that received saline or 2 mg/kg varenicline. Thirty min after ethanol injection, mice were retested. Again, a significant main effect of pretreatment was observed ($H_{3, N=63} = 9.2$, $p < 0.05$). Mice that received 1 or 2 mg/kg varenicline injection prior to ethanol challenge showed significantly more motor incoordination compared to mice that received a saline injection.

Fixed-Speed Rotarod

Varenicline had no effect on ethanol-induced ataxia when measured on the rotarod (Fig. 4). Varenicline had no effect on motor incoordination observed following a saline injection, thus to simplify the analysis we examined ethanol-induced ataxia when corrected by the mean levels of ataxia in the corresponding saline groups. When the corrected area under the curve (AUC) was analyzed with a Kruskal-Wallis ANOVA, there was not a significant effect of pretreatment with varenicline ($p=0.43$).

LORR

Varenicline increased the duration of LORR in C57BL/6J mice (Fig. 5). A 2-way ANOVA of the duration of LORR with sex and pretreatment dose as independent variables revealed a significant main effect of pretreatment dose ($F_{3, 76}=7.5$, $p<0.001$) and sex ($F_{1, 76}=11.6$, $p<0.01$; male mice had a longer LORR duration than female mice; 84.1 ± 3.9 vs. 65.0 ± 4.7 min, respectively), but no interaction of the two factors. Pretreatment with 1 or 2 mg/kg of varenicline increased the duration of ethanol-induced sedation compared to the saline group.

DISCUSSION

Recent studies have provided evidence that varenicline, a potent partial agonist of $\alpha 4\beta 2$ nAChRs with lower affinity for other nAChR subtypes (Coe et al., 2005; Mihalak et al., 2006; Rollema et al., 2007b), decreases ethanol consumption in rats, mice and humans (Kamens et al., 2010; McKee et al., 2009; Steensland et al., 2007). Here we show that varenicline modulates ataxic and sedative-hypnotic effects of an acute ethanol challenge as well. These data provide evidence that ethanol-induced ataxia and the sedative hypnotic effects of ethanol are modulated by nAChR activity.

The current set of experiments shows that varenicline increases ethanol-induced motor incoordination as measured by the balance beam and the dowel test. Ataxia is a complex behavioral phenotype, thus we used three different measures of ethanol-induced motor incoordination: the rotarod, balance beam and dowel test. Whereas varenicline increased ethanol-induced motor incoordination on the balance beam and dowel test, it had no significant effect on ethanol's ability to impair rotarod performance. While it is not yet clear why there is a difference in the effects of varenicline on these three tests, it is known that ataxia is a complex behavior and that no single measure of this trait can fully capture this behavior (Crabbe et al., 2005). It is likely that the balance beam and dowel test measure a different aspect of motor incoordination that is not captured by the fixed-speed rotarod test. Evidence for this comes from a study by Crabbe and colleagues (Crabbe et al., 2005), showing that there is a negative genetic correlation between performance on the fixed-speed rotarod at 0 minutes and dowel performance. In contrast there is a significant positive correlation between dowel and balance beam performance. From these studies we suggest that varenicline affects some aspect of ethanol-induced motor incoordination that is captured by the dowel test and balance beam.

Only one published study has provided evidence for a role of nAChRs in ethanol-induced motor incoordination. $\alpha 4\beta 2$ receptors in the cerebellum were implicated in ataxia as measured on the fixed-speed rotarod (Taslim et al., 2008). Taslim et al (2008) reported that intra-cerebellar administration of RJR-2403, an $\alpha 4\beta 2$ selective agonist, decreased ethanol-induced motor incoordination. In the current study we did not observe an effect of varenicline on impairment of rotarod performance by ethanol. We based the parameters of the task on the Taslim et al (2008) study to try to maximize the possibility of seeing an effect of varenicline, however there are a number of differences between these two studies that may explain the discrepancy in our findings. First, C57BL/6J mice were used here

whereas the previous study used CD-1 mice. Second, the RJR compound is a full agonist at $\alpha 4\beta 2$ nAChRs while varenicline is a partial agonist at these receptors. In addition, varenicline has activity at other nAChRs, while there is no evidence of actions of RJR-2403 at nAChR subtypes other than $\alpha 4\beta 2$ nAChRs. Finally, varenicline was administered peripherally in the current set of experiments but directly into the cerebellum in the Taslim, 2008 study. Therefore it is possible that the levels of varenicline in the cerebellum achieved in the current study may not have been high enough to alter ethanol-induced ataxia.

In the current experiments, the effect of varenicline on ethanol ataxia in the dowel test was dependent upon the testing time. Immediately after the ethanol injection the 0.5 mg/kg dose of varenicline increased ataxia compared to saline, but 30 min after the injection the higher doses of varenicline (1 and 2 mg/kg) increased ethanol-induced ataxia. One possibility that might explain this shift in the behavior from an early effect of a low dose to a later effect of the higher doses is that multiple nAChRs can modulate ethanol-induced ataxia as measured in the dowel test. Perhaps varenicline acts initially on a subset of nAChRs that are of higher affinity than those that are recruited 30 min after ethanol administration. Alternatively, it is possible that varenicline is being cleared at the 30 min time point, and that there is a very sharp inverted U function for the effects of varenicline, as has been seen for several behavioral effects of nicotine itself (Picciotto, 2003). Thus, blood levels of varenicline may be reached early after injection of the low dose and later after injection of the high doses. It is not possible to distinguish between these possibilities from the current dataset, but further dose-response and time course experiments could resolve this issue.

Varenicline also increased the duration of LORR, a common measure of ethanol's sedative-hypnotic effects. The role of the $\alpha 7$ nAChR subunit in this effect of ethanol has been examined previously using genetically modified mice. Mice lacking the $\alpha 7$ nAChR subunit were more sensitive to the sedative effects of ethanol compared to wild-type animals as evidenced by a longer LORR (Bowers et al., 2005). This would be consistent with previous studies showing that varenicline can bind to and activate $\alpha 7$ nAChRs (Mihalak et al., 2006; Rollema et al., 2007b). Taken together with the current finding that varenicline can increase the duration of LORR, these data suggest that both $\alpha 7$ and $\alpha 4\beta 2$ nAChRs may be important in this effect of varenicline on ethanol-induced LORR.

In the LORR experiment we observed a significant main effect of sex, such that male mice had a longer LORR duration compared to female animals. Importantly, sex did not significantly interact with varenicline dose, suggesting that the dose-response pattern was similar across both sexes. Sex differences in response to sedative doses of alcohol have been reported in the literature (Dudek et al., 1993), although the direction of effect is not consistent across studies, suggesting that males or females are not always more or less sensitive to this effect to ethanol. In addition, some studies have reported no sex difference in this behavioral response (Boehm et al., 2004). Thus, the sex differences in LORR may vary by species or genetic background in a complex manner.

In addition to effects of varenicline on alcohol related behaviors, we observed that 2 mg/kg varenicline had pronounced effects on baseline locomotor activity on its own in female C57BL/6J mice for the first 30 min after administration. There were no significant differences in locomotion during the epoch 40-50 min after the 2 mg/kg varenicline injection compared to saline, but activity remains lower. In male mice the duration of the depressant effect of 2 mg/kg is shorter and locomotor activity rebounds to the level of saline treated animals by 30 min after the varenicline injection. Moreover, 1 mg/kg varenicline does not have significant effects on locomotor activity compared to saline treatment. These data are not in agreement with locomotor activity studies performed in rats. In male Wistar rats, varenicline (0.3–3 mg/kg) increased baseline locomotor activity (Zaniewska et al., 2008).

The difference in varenicline-induced changes in baseline locomotor activity between rats and mice may not be surprising. Nicotine (a nAChR agonist) also has dramatically different effects on locomotor activity in rats versus mice. In rats locomotor activation is observed following an acute injection of nicotine (Elliott et al., 2005; Zaniowska et al., 2008), whereas in mice locomotor depression is most commonly observed (Marks et al., 1989; Tritto et al., 2004).

To avoid any confounds of locomotor depression, in the current experiments varenicline was always given 30 min prior to ethanol challenge. Based on both the dose-response function and the time-course of the locomotor effects of varenicline, the effects on ataxia and LORR do not appear to be due varenicline's effect on basal locomotor activity since locomotor activity approached baseline levels by the time of the ethanol challenge. Finally, varenicline had no effects on ataxia in the absence of ethanol challenge on any of the ataxia measures, thus all the significant effects observed were due to an interaction between varenicline and alcohol.

The effect of varenicline on ethanol behaviors also does not appear to be due to a change in the metabolism of ethanol since we have previously shown that varenicline does not alter ethanol-induced metabolism in C57BL/6J mice (Kamens et al., 2010), and varenicline had no effect on blood alcohol levels in humans (McKee et al., 2009).

Varenicline is a high affinity $\alpha_4\beta_2$ partial agonist (Coe et al., 2005; Rollema et al., 2007a) and binds with lower affinity to other nAChR subtypes including $\alpha_3\beta_2$, $\alpha_3\beta_4$, α_6^* and α_7 (Mihalak et al., 2006; Rollema et al., 2007b). The doses of varenicline that affect ethanol-induced behaviors in the current study (1–2 mg/kg) result in varenicline levels in rat brain high enough to have effects on multiple nAChR subtypes (Rollema et al., 2009). We have shown previously that varenicline at these doses decreased ethanol consumption, an affect that was not reversed by knocking out either the β_2 or α_7 nAChR subunit alone (Kamens et al., 2010). Thus, it is possible that these behavioral effects of ethanol might be modulated by other subtypes in addition to (or instead of) $\alpha_4\beta_2$ nAChRs.

In general, increasing doses of ethanol produce greater ataxia and LORR (Crabbe et al., 2003a; Crabbe et al., 2003b; Kamens et al., 2005). It is therefore possible to speculate that varenicline may shift the dose-response function of ethanol to the left by making animals more sensitive to lower doses of ethanol. Future studies that examine the effect of varenicline on a range of ethanol doses may address this possibility. nAChR may function to enhance sensitivity to ethanol and this may be one reason why mice pretreated with varenicline may have decreased ethanol consumption. It is possible that these negative effects of ethanol are felt after consuming less ethanol when pretreated with varenicline compared to when pretreated with saline.

Clinical studies have provided strong support for the role of varenicline as an effective smoking cessation aid (Cahill et al., 2008). Furthermore, there is accumulating evidence that this drug might also prove effective for treatment of alcohol abuse (McKee et al., 2009). Pre-clinical experiments that have examined the effect of varenicline on rats and mice have assessed the effects of the drug following chronic alcohol consumption (Kamens et al., 2010; Steensland et al., 2007), a treatment regimen that is relevant to the chronic ethanol intake that occurs in human alcoholics. In contrast, the current experiments examined the effect of varenicline on behaviors following acute alcohol administration. The current set of data suggests that the ability of varenicline to increase the adverse behavioral effects of ethanol may also contribute to its ability to decrease ethanol consumption, however it would be important to examine the effects of varenicline on ataxia and the sedative effects of alcohol following chronic ethanol administration to examine this hypothesis further.

There are currently three medications approved for the treatment of alcohol abuse: disulfiram, acamprosate, and naltrexone. At least two of these drugs have also been shown to increase negative effects of alcohol. In animal models acamprosate can enhance ethanol's sedative effects (Blednov and Harris, 2008). Moreover, in human clinical studies naltrexone can increase the sedative effects of alcohol (McCaul et al., 2000; Swift et al., 1994); however other studies have reported no change in this effect (King et al., 1997). Studies examining how varenicline alters the subjective response to alcohol in human subjects, including sedative effects, would be useful in testing the clinical relevance of the current findings.

Varenicline can attenuate the release of dopamine following acute administration of ethanol (Ericson et al., 2009) and it decreases the amount of responding for ethanol in a self-administration experiment (Steensland et al., 2007). These data provide support for the idea that varenicline may decrease the rewarding properties of alcohol. In addition, the current study suggests that varenicline can also exacerbate the ataxic and sedative effects of an acute alcohol challenge. While further studies will be necessary to determine whether an increase in the negative consequences of alcohol intake contributes to the clinical efficacy of varenicline in reducing drinking in human studies, the experiments described here provide additional support for the development of nicotinic agents to treat alcohol abuse.

Acknowledgments

These studies were performed with support from AA15632 (MRP), DA14241 (MRP), DA00436 (MRP), and MH014276 (HMK). The authors thank Dr. Hans Rollema for comments on an earlier version of this manuscript.

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Fig. 1.

Acute varenicline administration decreases locomotor activity. Data (mean $\pm$ SEM) represent locomotor activity of (A) female and (B) male mice following an acute injection of varenicline. N=7-8 per group. *, $p < 0.05$, significantly different from saline; #, $p < 0.05$, significantly different from 1 mg/kg varenicline.

Fig. 2.

Varenicline increases ethanol-induced motor incoordination as measured on the balance beam. Data (mean ± SEM) represent the number of footslips corrected by the average number of footslips of the corresponding saline group (saline: 0.43 ± 0.25 , 0.5 mg/kg: 0.5 ± 0.17 ; 1 mg/kg: 0.21 ± 0.11 ; 2 mg/kg: 0.21 ± 0.11). N=14-17 per group. *, $p < 0.05$.

Fig. 3. Varenicline increased ethanol-induced motor incoordination as measured in the dowel test. Data (mean $\pm$ SEM) represent the latency to fall from a 15.9 mm dowel (**A**) immediately after or (**B**) 30 min after a 1.5 g/kg ethanol injection. N=15-17 per group. *, $p < 0.05$.

Fig. 4. Varenicline does not alter ethanol-induced ataxia as measured on the rotarod. Data (mean $\pm$ SEM) represent 23-25 mice per group.

Fig. 5.

Varenicline increases ethanol-induced sedation as measured by LORR in C57BL/6J mice. Data (mean $\pm$ SEM) represent the length of time the mouse was unable to right itself after a 4 g/kg dose of ethanol. N=21 per group. *, $p < 0.05$.