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Title

The effects of long-term varenicline administration on ethanol- and sucrose-seeking and self-administration in male P rats.

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Abstract

Background: Varenicline, a partial agonist at α4β2 and full agonist at α7 nicotinic cholinergic receptors, is FDA approved for treatment of smoking cessation and has been found to reduce alcohol craving in clinical populations. In rodents, varenicline decreases free-choice ethanol intake with somewhat mixed findings in operant paradigms that utilize a combined appetitive/consummatory response.

Methods: The present experiment utilized an operant paradigm that procedurally separates appetitive from consummatory responding and a "reward blocking" approach (i.e., rats were able to consume ethanol during treatment) to better understand the efficacy of varenicline as a treatment for ethanol self-administration and subsequent ethanol-seeking. Separate groups of ethanol- and sucrose reinforced alcohol preferring, male P rats experienced alternating cycles of vehicle (2-week cycles) and varenicline (0.3, 1.0 and 2.0 mg/kg self-administered in a gelatin preparation) treatment (3-week cycles) prior to daily sessions where a single lever-press resulted in 20 minutes of reinforcer access. At the end of each cycle, a single extinction session assessed the seeking response in the absence of drug pretreatment.

Results: Varenicline dose-dependently decreased ethanol intake. Sucrose intake was largely unaffected, with no overall treatment effects and only sporadic days where the medium and high dose differed from vehicle. Neither sucrose nor ethanol-seeking was significantly decreased by varenicline, and there were no treatment effects on either lick or lever-press latency. Overall effect sizes were much greater for both drinking and seeking in the Ethanol Group as compared to the Sucrose Group.

Conclusions: Varenicline effectively attenuates ethanol self-administration during treatment, but the experience with ethanol consumption while varenicline is "on board" is not sufficient to alter subsequent ethanol-seeking. The overall pattern of findings indicate that varenicline blocks the rewarding properties of ethanol while not substituting for ethanol, that the non-specific effects on an alternate reinforcer are negligible, and that blood levels of varenicline need to be maintained in order for treatment to remain effective.

Introduction

Varenicline, a partial agonist at α4β2 and full agonist at α7 nicotinic cholinergic receptors, is FDA approved for treatment of smoking cessation and has been reported to be "more effective and satisfactory" by patients when compared with bupropion and nicotine replacement therapy, both of which are also approved for smoking cessation (Etter and Schneider, 2013). As reviewed by Aubin et al. (2014), varenicline has the largest effect size of the approved treatments and extended treatment with varenicline may prevent relapse to smoking. Serendipitous findings indicated that alcohol consumption was also decreased in individuals receiving varenicline treatment for smoking, and subsequent placebo-controlled clinical trials, one open-label study (Erwin and Slaton, 2014), and a large double-blind multi-site treatment trial (Litten et al., 2013) found varenicline to be efficacious at decreasing alcohol intake and alcohol craving. A more recent study confirmed that varenicline decreased alcohol craving, but did not find a decrease in alcohol drinking relative to placebo (de Bejczy et al., 2015).

In preclinical models, varenicline disrupts the discriminative effects of ethanol (Randall et al., 2015), decreases home-cage and fixed-ratio reinforced ethanol intake (Steensland et al., 2007), and blocks reinstatement to ethanol seeking (Funk et al., 2016). Systemic varenicline stimulates release of dopamine in the nucleus accumbens and direct administration of varenicline into the accumbens core decreases ethanol intake (Feduccia et al., 2014). While varenicline does not block ethanol-induced conditioned place preference, it decreases ethanol-induced locomotor stimulation

indicating that it does interact with some interoceptive effects of ethanol (Gubner et al., 2014), but importantly, it does not substitute for ethanol (Randall et al., 2015). Prior research has repeatedly shown that orally self-administered varenicline decreases home-cage ethanol intake in alcohol-preferring P rats: 1) following long term administration when it tested with a one-hour pretreatment time (Froehlich et al., 2016); 2) if it is given with the first opportunity to drink ethanol but not if it is given as a preventative treatment prior to onset of the first experience with ethanol (Froehlich et al., 2017a); and 3) in cases when escalation in intake would normally be seen following ethanol deprivation and alcohol re-access (Froehlich et al., 2017b).

The present study sought to extend these previous findings using our operant paradigm that was designed to model the experience of a heavy drinking individual on long-term treatment. Specifically, rats experienced three-week long cycles of varenicline treatment and daily operant sessions with a low response requirement that resulted in access to ethanol. At the end of each varenicline treatment cycle, the rats were tested in a single extinction session to assess their seeking response. We have previously used this seeking/drinking operant paradigm to demonstrate that naltrexone, one of the three drugs approved by the FDA for treating alcohol use disorders, selectively reduces ethanol-seeking as compared to sucrose-seeking in nondependent Long Evans rats (Czachowski and DeLory, 2009). Interestingly, evidence suggests that naltrexone treatment may be more efficacious in patients who continue to drink some alcohol during treatment (Heinälä et al., 2001; Killeen et al., 2004). The interaction between consumed ethanol and naltrexone's efficacy may be partially explained by a learned, associative component of a "disruption" of ethanol reward while continuing to drink during treatment (Stromberg et al., 1998). The goal of the present study was to determine the efficacy of varenicline to reduce ongoing ethanol drinking as well as to reduce ethanol "craving-like" behavior in animals experienced with drinking ethanol during varenicline treatment. To examine the specificity of varenicline's effects, sucrose was used as an alternate reinforcer in a separate group of animals.

Materials and Methods

Subjects

Subjects were alcohol-naïve male P rats from the 79th generation of selective breeding for alcohol preference (obtained from the Indiana University School of Medicine, Indianapolis, IN) that were randomly assigned to either an ethanol-reinforced group or a sucrose-reinforced group (n=12/group). Ad libitum access to food and water was maintained throughout the experiments except where noted below. Training began at 8 weeks of age (weights: 208 +/-15 g). Animals were individually housed on a 12-h light/dark cycle (5:00 a.m. to 5:00 p.m.), and all animal care procedures were conducted in accordance with the NIH Guidelines for the Care and Use of Laboratory Animals (2011), and all protocols were approved by the Institutional Animal Care and Use Committee (IACUC).

Apparatus

Daily sessions were conducted in modular chambers (Med-Associates; St. Albans, VT, USA; 30×30×24.5 cm) equipped with a houselight, a retractable lever, and a retractable graduated cylinder tube with rubber stopper and a stainless steel spout with double ball bearings to prevent leakage. The lever was located on the wall opposite to the sipper tube drinking bottle. All chambers were housed in sound-attenuated enclosures equipped with exhaust fans that masked external noise. Electrical inputs and outputs of each chamber were controlled using Med-Associates software (Med-Associates).

Drugs

Ethanol solutions were prepared volume/volume in water from 95% ethanol. The sucrose/ethanol solutions were prepared weight/volume using sucrose as the solute in the ethanol solution. Varenicline (provided by Pfizer, Inc., USA) was incorporated into flavored star-shaped pieces of gelatin that were voluntarily consumed by the rats. Varenicline was added to a sweetened

gelatin solution comprised of berry flavored Jell-O, gelatin, dextrose, sodium saccharin and Magnasweet in distilled, deionized water. The gelatin solution, containing drug or no drug (vehicle), was aliquoted into star shaped molds, one per rat per day, with the volume of each aliquot determined by the body weight of the rat, as previously described (Froehlich et al., 2013; 2016; 2017). Gelatin stars (approximately 1.8 gram) were fed to the rats once each day inserted through the wire top of their home cage. The rats consistently ate the gelatin within 1-2 minutes. Cages were checked to confirm that no pieces of gelatin were uneaten. On treatment days, gelatin stars in doses of 0.3, 1.0 or 2.0mg/kg were administered at 1 hour prior to onset of operant sessions.

Training and Ethanol Initiation

Upon arrival, subjects were weighed (start weights: 208 +/-15 g) and handled for a minimum of 3 days prior to onset of operant sessions. Daily sessions were conducted 5 days/week at the same time each day during the light portion of the light-dark cycle. Subjects were initially trained to press the lever on a fixed ratio-one schedule that resulted in 15 seconds of access to the sipper tube with 10% sucrose in 30-minute sessions. Subjects were water-restricted for the initial two to five sessions only, after which food and water were available ad libitum in the home cage. Over a 3-week period, the training/sucrose-fading (Samson, 1986) procedures involved increasing the fixed ratio from 1–4, decreasing the sucrose concentration to 1% and, in the ethanol groups, introducing ethanol and increasing the concentration from 2% to 10% while fading the sucrose out completely to produce final solutions of either 1% Sucrose or 10% Ethanol. The procedural separation between seeking (lever pressing) and consumption was then instituted. Following completion of the single response requirement, access to the sipper tube was provided for 20 minutes. Over 4 weeks, the response requirement was increased from 4 lever-presses to 20, and then the response requirement of 20 was maintained for an additional week. In order to habituate the rats to the procedure for oral administration of drug, subjects were fed gelatin stars containing no drug (vehicle) during the final week of training on two occasions, after that day's behavioral session.

Treatment Schedule and Test Sessions

The experimental design was similar to the design we have previously used to examine the effects of naltrexone and acamprosate in nondependent Long Evans rats (Czachowski and DeLory, 2009). Following training, there were alternating daily session treatment "cycles" of drug (a total of 3 weeks or 14 total sessions from a Tuesday to Friday) and vehicle [a total of 2 weeks or 9 total sessions from a Tuesday to Friday (vehicle exposure was shorter than varenicline exposure since no tolerance or sensitization to vehicle was predicted)]. For these daily sessions, the response requirement was decreased to a single lever-press to ensure that all subjects would gain access to the reinforcer solution (i.e., they would be unlikely to "fail" the response requirement). At the end of each three-week drug and two-week vehicle treatment cycle, a single, non-reinforced extinction session was conducted on a Monday (i.e., following 2 days without drug treatment or access to the reinforcer solution). Extinction sessions consisted of 20 minutes of access to the lever, with no presentation of the sipper tube spout (however, solutions were present in the sipper tube to control for scent cues). The response requirement was then gradually increased back to 20 for the remainder of that week (no treatments) through the following Monday to ensure subjects would continue to reliably respond (i.e., that the prolonged exposure to the single response requirement schedule did not cause a decrement in responding), and then the next cycle was initiated (alternating vehicle and drug). Each drug treatment cycle consisted of a single dose of varenicline assigned to each animal in a balanced design which was repeated 3 times, with the dose changed each time, so that at the end of three drug cycles all doses had been tested in all animals. The entire experiment lasted a total of 192 days.

Statistical Analyses

Total intakes of sucrose and ethanol were determined from the change in fluid volume in the graduated cylinder sipper tube, and grams per kilogram of intake were calculated from the intake volume, concentration and daily body weight measures. Total lever-presses as well as a cumulative

record of responding were recorded for each session. For intake, ethanol-reinforced and sucrose-reinforced groups were analyzed separately (since g/kg differences between ethanol and sucrose would be driven by the differences in solution concentration) using repeated measures analysis of variance (RM ANOVA), and post hoc comparisons were performed using a Tukey test when appropriate. For seeking, two-way RM ANOVA was used to analyze lever-press responses. For intake data, Dose (four: vehicle plus three drug doses) and Day were the main factors over the first 9 days. In addition, mean intake collapsed over the nine (vehicle) or 14 (drug) day treatments was analyzed with Dose as the main factor. For the seeking data, Dose and Reinforcer were the main factors. In both groups, the two vehicle treatment cycles were collapsed for each subject (after confirming no differences with paired t-test, see below). Effect sizes were also calculated for mean g/kg intake and mean extinction responding for each dose of varenicline in each reinforcer group.

Results

In the Sucrose Group, there were 3 incidents of the sipper tubes air-locking (more likely with sucrose than ethanol due to the viscosity of the solution) in 3 different subjects and conditions. Their intake data for those days were calculated from the mean of the 2 preceding days (which differed by 0.02 g/kg or less). One subject in the Sucrose Group died with just 5 days remaining in the experiment (with no obvious cause) and 2 different subjects in the Sucrose Group did not consistently finish consuming the gelatin star (one at the moderate dose and one at the high dose), so these three subjects were removed from the data analyses. In the Ethanol Group, 2 different animals failed to complete the response requirement on a single day (one in the low and one in the moderate dose condition) and the remaining 13-day mean was used for the missing data. One subject failed to respond for 3 consecutive days after breaking a toe-nail before the session, and the 11 remaining days were used for the missing data. There were no missing extinction data points for the Ethanol Group. Final group sizes were 9 for the Sucrose Group and 12 for the Ethanol Group.

As stated above, t-tests confirmed no differences in the Sucrose or Ethanol Groups for any of the intake or seeking measures from the first to the second vehicle treatment cycle, so those data were collapsed for a single vehicle measure. In the ethanol-reinforced group, analysis of ethanol intake (g/kg) following vehicle or varenicline over days 1–9 (Fig. 1) showed that there was a main effect of Dose [$F(3,264)=11.2$, $p<0.001$] with post hoc analyses indicating that the medium and high doses differed from vehicle, and the low and high doses differed from each other. There was also a main effect of Day [$F(8,264)=3.2$, $p<0.01$] and a Day/Dose interaction [$F(24,264)=2.2$, $p<0.001$]. Post hoc analyses indicated that the effect of Day was due to increased overall intake on Day 5 relative to Days 1, 6 and 9 (likely a slight "Monday" rebound effect following the two days of no sessions – Day 5 was the only Monday drinking session for all groups/conditions). The Day/Dose interaction showed that there were no differences in intake across treatments on Days 1 or 2, however, on Days 3-5 intake following all varenicline doses was significantly lower than following vehicle. The high dose continued to differ from vehicle over the remaining Days, and also differed from the low dose on Days 5-9 and the medium dose differed from vehicle again on Day 8. Analysis of total mean ethanol intake collapsed over all days similarly showed a main effect of Dose [$F(3,33)=14.$, $p<0.001$], with post hoc analyses confirming that both the medium and high doses differed from vehicle and that the high and low doses were also significantly different from each other.

In the sucrose-reinforced group, analysis of sucrose intake (g/kg) following vehicle or varenicline (Fig. 2) over days 1–9 showed no main effect of Dose [$F(3,192)=1.1$, $p=0.37$]. However, there was a main effect of Day [$F(8,192)=5.7$, $p<0.001$] and a Day/Dose interaction [$F(24,192)=3.1$, $p<0.001$]. Post hoc analyses indicated that the effect of Day was due to an overall decrease in intake over days (Day 1 vs. 5, 6, 8 and 9, Day 2 vs. 9, Day 3 vs. 8 and 9). However, the Day/Dose interaction showed that when Days within Dose were examined, intake was stable across the vehicle treatment. There were only sporadic effects of varenicline when examined within Days, such that there was a significant effect of the high dose of varenicline relative to vehicle on Days 1, 6 and 9. On Day 6, the medium dose and vehicle also differed, while there were no differences between the

low dose and vehicle on any Day. Analysis of total mean sucrose intake collapsed over all days showed no main effect of Dose [F(3,24)=2.2, p=0.12].

Analysis of the seeking response assessed lever-presses on the extinction test days and revealed no difference in number of responses between the Sucrose and Ethanol Groups [F(1,57)=1.2, p=0.28], no main effect of Dose [F(3,57)=1.9, p=0.15] and no interaction between Reinforcer and Dose [F(3,57)=0.5, p=0.70]. There was also no difference in latency to first lever-press between the sucrose and ethanol Reinforcers [F(1,57)=1.1, p=0.30], no main effect of Dose [F(3,57)=0.2, p=0.89], and no interaction between Reinforcer and Dose [F(3,57)=0.3, p=0.86]. Similarly, an analysis of the average latency to first lick on the intake treatment days confirmed that there was also no difference between the sucrose and ethanol Reinforcers [F(1,57)=1.6, p=0.22], no main effect of Dose [F(3,57)=1.4, p=0.24], and no interaction between Reinforcer and Dose [F(3,57)=0.7, p=0.55].

Overall effect sizes were larger in the Ethanol Group than in the Sucrose Group, and in fact were nearly nonoverlapping between Groups within both the drinking and seeking responses across varenicline doses (see Table 1).

Discussion

The behavioral paradigm used here presented rats with long-term (3 weeks) exposure to ethanol consumption with varenicline “on board”, and then tested the effects of this experience on subsequent ethanol-seeking as a measure of “craving” at a time when varenicline would no longer have been pharmacologically active. Importantly, the model also separates seeking and drinking for distinct measures of each reinforcer-directed behavior, and the self-administration of varenicline by the oral route avoids the stress effects of multiple injections. Varenicline is well absorbed after oral administration and has a bioavailability of greater than 87% (Goodman and Gilman, 2011; Obach et al., 2005). While it is primarily considered a partial agonist at α4β2 and full agonist at α7 nicotinic cholinergic receptors, it should be noted that varenicline also has agonist activity at serotonin (5HT3)

receptors (Lummis et al., 2011; Price et al., 2015). It has previously been shown that a 2.0 mg/kg dose of oral varenicline at 1 hour prior to onset of ethanol access (-2hr pretreat was less effective) decreases free access intake of ethanol in male P rats (Froehlich et al., 2016). The present findings extend these observations and illustrate that the effect of varenicline on ethanol intake is both dose-dependent and selective for ethanol self-administration. Specifically, 0.3, 1.0 and 2.0 mg/kg doses decreased ethanol self-administration over several individual days of testing. This effect emerged on day 3 for all doses, and then persisted though the remaining test days for the medium and high doses. This effect was also apparent in the collapsed measure of ethanol consumption across all days.

The fact that the first two days of treatment resulted in no decrement in self-administration suggests that, like naltrexone, the effects of varenicline may be related to its ability to interfere with the rewarding properties of ethanol. This apparent "reward-blocking" effect of varenicline is consistent with a recent report in humans that consumed high doses of alcohol during varenicline treatment and reported greatly reduced subjective intoxication that was dose-dependent and long-lasting (Verplaetse et al., 2016a). Our findings are also consistent with those of Randall et al. (2015) who found no effect of a single, acute administration of varenicline on operant responding in Long Evans rats given doses of varenicline that did not interfere with motor performance (i.e., 0.3 and 1.0 mg/kg). Notably, the reinforcer in that paradigm was sweetened ethanol (2% sucrose/15% ethanol) and the operant response was a fixed ratio of two responses (FR2), so it was a "mixed" seeking/drinking response unlike the purely drinking response utilized in the present study. Steensland et al. (2007), on the other hand, showed that both a 1.0 and 2.0 mg/kg dose were effective in decreasing operant responding for ethanol even on the first day of acute treatment, however the operant paradigm required three lever presses for a "sip" of ethanol. Attenuation of ethanol drinking in the present study is not likely to be due to any motor impairing effects of varenicline, since there were no differences between vehicle and treatment in the time to first lever-

press or the time to first lick (these are not explicitly measures of locomotion, but making the first lick does require the animal to traverse to the opposite side of the operant chamber).

Overall, there was no attenuation of sucrose intake by varenicline when analyzed across all days of testing. There were only sporadic effects of the medium and high doses on specific days that did not suggest any pattern of varenicline efficacy. Using Cohen's (1969) general characterization of effect sizes of 0.2 as "small", 0.5 as "medium" and 0.8 as "large", the effect of varenicline on sucrose drinking was in the medium range (0.34-0.74 over all doses) whereas the effect size for ethanol drinking was in excess of large (0.67-1.81 over all doses). These results are consistent with, and extend the findings of, Steensland et al. (2007) by showing specific effects of varenicline on a mixed seeking/drinking response for ethanol and not for sucrose. The present results are also consistent with the recent findings of Holgate and colleagues (2017) who showed that varenicline decreased free-access intake of ethanol to a much greater degree and for a longer period of time as compared to sucrose.

The tests of reinforcer-seeking were conducted in animals with reinforcer-plus-varenicline exposure, but with no varenicline currently "on board". The expectation was that sufficient exposure to pairings of reinforcer-plus-varenicline would impact overall subjective reinforcer effects, ideally specifically ethanol but not sucrose, and thereby decrease subsequent reinforcer-seeking indicating a reduction in "craving". Overall, we found that the effects of varenicline on the response/reinforcer association were not strong enough to interfere with goal-directed responding after the termination of varenicline treatment. It has previously been shown that a high dose of varenicline has some carry over effects in that it has a tendency to decrease 2-hour free choice ethanol intake one day following cessation of treatment (Froehlich et al., 2016), but these effects were not apparent two days later. Notably, in that study, intake of ethanol on that first day in the absence of varenicline would have given subjects exposure to ethanol alone which would be expected to facilitate a return to normal drinking.

In the present study, there were some indications that varenicline had specific effects on ethanol-seeking. There was some decrement of ethanol-seeking across all doses (an average of 72-83% relative to vehicle as compared to 82-93% in the Sucrose Group), however, this was not sufficient to reach statistical significance. On the other hand, effect sizes indicate a low to medium effect of varenicline on sucrose seeking (0.30-0.59) and a medium to large effect on ethanol-seeking (0.53-0.82). Note that Randall et al. (2015) showed that varenicline does not substitute for ethanol and our data support that conclusion. The decrease in ethanol drinking we observed is not due to varenicline substituting for, or enhancing, the reinforcing properties of ethanol, since subsequent reinforcer-seeking does not increase, but actually decreases slightly. Taken together, our findings show that varenicline blocks some rewarding properties of ethanol, but that it is important for varenicline to be "on board" to observe strong and sustained effects of the drug on subsequent ethanol craving. This is consistent with recent findings in humans showing that lower doses of varenicline do not decrease alcohol craving as effectively as higher doses and that there is a direct relationship between plasma levels of varenicline and number of drinks consumed (Verplaetse et al., 2016b). Given that varenicline is well-tolerated, that previous concerns regarding potential cardiovascular effects have been disproven (Aubin and Luquiens, 2015; Prochaska and Hilton, 2012), and that both the preclinical and clinical evidence continues to support the fact that varenicline is effective at decreasing ethanol-directed behaviors, it appears to be a good pharmacotherapeutic tool for treating alcohol use disorders at this time. Moreover, optimal combinations of varenicline with other medications may be the ultimate solution for targeting alcohol-specific craving and binge drinking (e.g., Froehlich et al., 2016).

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Figure Legends

Fig 1. In the ethanol-reinforced group (n=12), mean (±SEM) ethanol intake (g/kg) over days following either vehicle or varenicline treatment (data from days preceding the dotted line were analyzed by Day and Dose). Inset: Total mean (±SEM) ethanol intake collapsed over all days. One asterisk indicates a main effect of treatment and significant difference relative to vehicle, two

indicate a difference relative to both vehicle and the low dose. See text for specific effects of doses on individual days.

Fig 2. In the sucrose-reinforced group (n=12), mean (±SEM) sucrose intake (g/kg) over days following either vehicle or varenicline treatment (data from days preceding the dotted line were analyzed by Day and Dose). Inset: Total mean (±SEM) sucrose intake collapsed over all days. See text for specific effects of doses on individual days.

Fig. 3. Mean (±SEM) lever-press responses during single, nonreinforced extinction sessions in the ethanol and sucrose groups (as indicated) following prior exposure to vehicle or varenicline during reinforced sessions.

Table 1.

Mean latency to first lick and latency to first lever press in seconds (±SEM) listed by increasing dose (0, 0.3, 1.0 and 2.0 mg/kg varenicline) for the Ethanol and Sucrose Groups.

	Lick Latency (± SEM)	**Lever-Press Latency (± SEM)**
Ethanol Group	4.2 (1.6) 5.3 (1.8) 5.5 (1.7) 21.0 (14.5)	67.3 (41.6) 40.5 (15.9) 47.1 (23.2) 64.4 (47.4)
Sucrose Group	1.6 (0.4) 1.3 (0.1) 1.6 (0.2) 4.4 (2.1)	24.1 (6.4) 23.2 (6.5) 19.2 (8.1) 16.1 (6.1)

Table 2.

Varenicline effect sizes for each behavior (drinking and seeking) listed by increasing dose (0.3, 1.0, 2.0) for the Ethanol and Sucrose Groups.

	Drinking	**Seeking**
Ethanol Group	0.67, 1.21, 1.81	0.82, 0.71, 0.53
Sucrose Group	0.52, 0.34, 0.74	0. 30, 0.59, 0.42