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Desipramine enhances the ability of paliperidone to decrease alcohol drinking

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Abstract

Alcohol use disorder commonly occurs in patients with schizophrenia and dramatically worsens their course. The atypical antipsychotic clozapine has been associated with reduced drinking in these patients, but its toxicity reduces its use. We have attempted to create a clozapine-like drug by combining agents that capture components of clozapine's pharmacologic action, including its weak dopamine D2 blockade and noradrenergic modulation. The current study assessed whether paliperidone, a dopamine D2 receptor and adrenergic alpha-2 receptor antagonist like clozapine, would attenuate alcohol drinking in the alcohol-preferring P rat and the Syrian golden hamster, and whether desipramine, a norepinephrine reuptake inhibitor, would potentiate the ability of paliperidone to attenuate alcohol drinking in the P rat and the Syrian golden hamster. Daily subcutaneous injections of paliperidone (5 mg/kg for the rat; 1 mg/kg for the hamster) over 20 days slightly and transiently attenuated initiation of alcohol consumption in both animals. Desipramine (3 mg/kg) or lower doses of paliperidone alone did not affect alcohol drinking. However, the combination of desipramine (3 mg/kg) and paliperidone essentially prevented initiation of alcohol drinking and acquisition of alcohol preference in the P rat (2.5 or 5 mg/kg), and almost as dramatically suppressed chronic alcohol intake and alcohol preference in the hamster (2.5 mg/kg). Taken together, the current data suggest that (1) the desipramine and paliperidone combination attenuates alcohol drinking in a synergistic manner, and (2) desipramine and paliperidone may serve as an effective new treatment for alcohol use disorder in patients with schizophrenia.

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INTRODUCTION

Alcohol use disorder (AUD) occurs in over one-third of patients suffering from schizophrenia (SCZ), which is threefold higher than AUD's prevalence in the general population (Drake et al. , 1989, Regier et al. , 1990). Patients with schizophrenia tend to consume moderate quantities of alcohol on a regular basis without developing physical withdrawal symptoms upon cessation of drinking, and tend to display alcohol abuse more frequently than alcohol dependence (Drake, Osher, 1989, Lehman et al. , 1996, Test et al. , 1989). However, even moderate use of alcohol is known to worsen the clinical course of schizophrenia (Alterman et al. , 1981, Drake and Mueser, 1996, Gupta et al. , 1996, Owen et al. , 1996). Our group and others have consistently shown that treatment of patients with schizophrenia using clozapine is associated with substantially lower rates of alcohol and drug abuse in this population (Drake et al. , 2000, Green et al. , 1999, Machielsen et al. , 2014). We have proposed that clozapine's unique and broad-ranging pharmacological profile, including its weak blockade of the dopamine D2 receptor, its potent antagonism of the noradrenergic alpha-2 receptor, and its ability to greatly and chronically elevate levels of norepinephrine may be responsible for its anti-alcohol abuse property (Green, Zimmet, 1999).

Risperidone is an atypical antipsychotic with pharmacologic properties partially overlapping with those of clozapine. Like clozapine, risperidone is an antagonist of dopamine D2 receptors and noradrenergic alpha-2 receptors (Richelson and Souder, 2000). Its metabolite paliperidone (9-OH-risperidone) has similar affinity for alpha-2 receptors but lower affinity (and rapidity of binding) for D2 receptors than risperidone (Leysen et al. , 1994, Richelson and Souder, 2000, Schotte et al. , 1995), making it more clozapine-like in its actions on these receptors.

Risperidone, however, may differ from clozapine in regard to norepinephrine reuptake, since it does not appear to elevate norepinephrine levels in patients with schizophrenia to the same extent as clozapine (Elman et al. , 2002, See et al. , 1999). We suggest that this difference between the clozapine and risperidone may explain why risperidone does not appear to limit alcohol drinking in patients with schizophrenia (Green et al. , 2008, Smelson et al. , 2002). In support of this suggestion, we have shown that while risperidone itself has little effect on alcohol drinking in the Syrian golden hamster, its co-administration with the norepinephrine reuptake inhibitor desipramine chronically suppresses alcohol drinking (Gulick et al. , 2014). The effect of paliperidone on levels of norepinephrine is less clear. In this regard, however, we note that one study indicated that risperidone, and not paliperidone, inhibited the firing of serotonergic neurons; moreover, this ability of risperidone to inhibit serotonergic neurons was antagonized by the addition of the norepinephrine reuptake inhibitor desipramine, suggesting that paliperidone may potentially have some norepinephrine reuptake inhibitor-like effects *in vivo* (Dremencov et al. , 2007).

We tested the ability of paliperidone alone, or in combination with desipramine, to reduce alcohol drinking, in two models of alcohol drinking--the alcohol preferring rat (P rat) and the Syrian golden hamster. These two animal models were chosen to represent two distinct stages of alcohol use disorder in patients with schizophrenia. As we have done previously,

we selected the alcohol-preferring P rat to model the initiation of alcohol drinking, and we used the Syrian golden hamster model chronic alcohol drinking. The P rat differs from patients with SCZ in that it develops high blood alcohol levels and physiologic dependence (McBride et al. , 2014). Moreover, our previous research has shown that clozapine, but not haloperidol, impairs initiation of alcohol drinking, but not maintenance of alcohol drinking in the P rat (Chau et al. , 2013), suggesting that this animal model only has predictive validity for patients with SCZ under the initiation phase. The hamster models chronic alcohol drinking in patients with schizophrenia for the following reasons: (a) like patients with schizophrenia who tend to drink regular but moderate amounts of alcohol, the hamster consumes alcohol on a regular basis, and does not develop physiologic withdrawal (Ferris et al. , 1998, Harris et al. , 1979, Keung et al. , 2000); and (b) like patients with schizophrenia, clozapine, but not haloperidol, reduces alcohol drinking in this animal (Green et al. , 2004). Using these models, we tested how paliperidone or paliperidone plus desipramine would alter the initiation of alcohol drinking (P rat) or chronic alcohol drinking (hamster), with an eye toward developing safer clozapine-like drugs for use in patients with schizophrenia and alcohol use disorders.

MATERIALS AND METHODS

Animals

All experiments were carried out in accordance with the National Research Council of the National Academies guide for the care and use of laboratory animals (Eighth Edition, National Academies Press 2011) and were approved by the Dartmouth Institutional Animal Care and Use Committee. Animals were maintained on a normal 12 h/12 h light/dark cycle, and individually housed in standard home cages with ad libitum access to food and water. A technician, blinded to the experimental conditions, measured fluid intake every 24 hours, and food intake and body weight every 3 days. Within each experiment, the positions of the two drinking bottles were rotated on a daily basis to prevent positional preference. All animals continued to receive free access to food, water and alcohol (24 hour) during the treatment period.

P Rats

Adult, male alcohol-preferring P rats, (320-520 g) were generously provided by the Indiana University Animal Research Center (Indianapolis, IN). Alcohol (10%, v/v) was presented as a second choice of fluid on specific days and at times indicated in the experimental protocols.

Hamsters

Adult, male Syrian golden hamsters (*Mesocricetus Auratus*) (100-130g) were acquired from Harlan Inc. (Indianapolis, IN). For each experiment, hamsters were given free access to a water bottle, a bottle containing alcohol (15%, v/v), and food. Once alcohol intake reached a steady baseline (which required two weeks of alcohol access on average), drug treatment began.

Experimental Protocols

Experiment 1: Paliperidone dose-response in the P rat—Forty-two rats were given free access to water and food and then randomized into 6 groups (n=7 per group) with similar baseline water intake values (ml) and body weight (g). The groups were treated subcutaneously daily for 4 days with either: VEH or one dose of paliperidone (PAL; 0.05, 0.2, 0.5, 1, 5 mg/kg). After the pretreatment phase, a bottle containing 10% alcohol was added to each cage, and drug treatment continued for an additional 20 days. The ability of the drugs to prevent the initiation (development) of escalated drinking was measured in all P rat experiments.

Experiment 2: High-dose paliperidone plus desipramine in the P rat—To test whether norepinephrine reuptake blockade could enhance the effects of paliperidone on the initiation of alcohol drinking, we tested whether desipramine (DMI) could potentiate the effects of a high dose of PAL on initiation of free access alcohol drinking following 4 days of drug pretreatment in the P rat. Forty-four rats were given free access to water and food and then randomized into 6 groups (n=7 per group) with similar baseline water intake values (mL) and body weight (g). The groups were subsequently treated daily for 4 days with either: VEH, paliperidone (2.5 or 5 mg/kg), DMI (3 mg/kg), or combinations of PAL with DMI. After the pretreatment phase, a bottle containing 10% alcohol was added to each cage, and drug treatment continued for an additional 20 days.

Experiment 3: Low dose paliperidone plus desipramine in the P rat—Because of the dramatic results for Experiment 2, we also tested whether DMI could potentiate the effects of a low dose of PAL on free access alcohol drinking following 5 days of drug pretreatment in the rat. Forty-eight rats were given free access to water and food and then randomized into 6 groups (n=7-8 per group) with similar baseline water intake values (mL) and body weight (g). The groups were subsequently treated daily for 5 days with either: VEH, paliperidone (0.5-1 mg/kg), DMI (3 mg/kg) or combinations of DMI with PAL. After the pretreatment phase, a bottle containing 10% alcohol was added to each cage, and drug treatment continued for an additional 20 days.

Experiment 4: Paliperidone dose-response in the hamster—Forty-two hamsters were given access to separate bottles of water and 15% v/v alcohol for 15 days prior to randomization into 5 groups (n=8-9 per group) with similar baseline alcohol intake values (g/kg). Baseline alcohol intake was calculated using the last 4 days of the initial access to alcohol. The groups were subsequently treated subcutaneously daily for 20 days with either: vehicle (VEH) or one dose of paliperidone (PAL) (0.05, 0.2, 0.5, or 1 mg/kg). A lower range of doses was used in the hamster because in previous experiments the P rat has required higher dose levels of clozapine than the hamster to achieve an effect on alcohol drinking (Chau, Khokhar, 2013). The ability of the drugs tested to impair chronic alcohol drinking was assessed in all hamster experiments.

Experiment 5: High-dose paliperidone plus desipramine in the hamster—We next examined whether DMI could potentiate the effects of a high dose of PAL (2.5 mg/kg; higher doses in the hamster may result in too potent of a dopamine D2 antagonism and

thereby diminish PAL's ability to reduce alcohol drinking) in the hamster, as it did in the P rat. Thirty hamsters were given access to separate bottles of water and 15% v/v alcohol for 27 days prior to randomization into 4 groups (n=7 per group) with similar baseline alcohol intake values (g/kg). The groups were subsequently treated daily for 20 days with either: VEH, paliperidone (2.5 mg/kg), DMI (3 mg/kg), or the combination of PAL with DMI.

Data Analysis

Alcohol intake (g/kg), alcohol preference (alcohol intake [mL]/total fluid intake [mL]), water intake (mL), food intake (g/kg), and body weight (g) data were analyzed using two-way repeated measures analysis of variance (RMANOVA), using time (day) and group (drug treatment) as independent variables. When the analysis indicated that significant differences existed between treatments, pairwise comparisons between groups were made using the Tukey adjustment. The pairwise comparisons were tested at each day to help interpret group $\times$ time interactions from the RMANOVAs; adjustment to p-values was carried out separately at each day. In the absence of an interaction, comparisons were also performed separately on the overall time effect when significant. Significance in all cases was determined at $p < 0.05$. Data are expressed as the group mean.

RESULTS

Experiment 1: Paliperidone dose-response in the P rat

Alcohol Intake—The RMANOVA results for daily alcohol intake in the rat during the 20-day treatment period indicated the presence of a significant effect of time, $F(19,684)=31.471$, $p < 0.001$ and of group, $F(5,36)=5.529$, $p < 0.01$ and a significant time by group interaction $F(95,684)=1.316$, $p < 0.05$. Post hoc tests further revealed that PAL dose-dependently attenuated initiation of alcohol drinking (Figure 1A). A 5 mg/kg dose of PAL was required to reduce alcohol drinking in the P-rat; no other doses of PAL differed from VEH on any treatment day. However, this reduction was transient over time, such that by day 13, rats treated with PAL drank similar amounts of alcohol as those treated with VEH ($p < 0.05$).

Alcohol Preference—The RMANOVA analysis of alcohol preference showed a significant effect of time, $F(19,684)=19.688$, $p < 0.001$, of group, $F(5,36)=4.705$, $p < 0.01$ and no time by group interaction $p < 0.001$. Tukey (post hoc) tests on the main effect of group demonstrated that PAL also dose-dependently lowered alcohol preference ($p < 0.05$) (Figure 1B). A significant linear overall Like alcohol intake, trend was indicated by the post hoc tests on the main effect of time ($p < 0.005$).

Water and Food Intake and Body Weight—The RMANOVA analysis of water intake produced a significant effect of time, $F(19,684)=31.758$, $p < 0.001$, and of group, $F(5,36)=2.602$, $p < 0.05$, and a significant time by group interaction $F(95,684)=1.508$, $p < 0.005$. Post hoc analysis showed that the highest PAL dose (5 mg/kg) group drank more water than VEH on days 1, 6-8, 11-12, ($p < 0.05$). Differences in water intake between the highest PAL dose and the two lowest (0.5 mg/kg and 1 mg/kg) were similar to those for VEH ($p < 0.05$). In contrast to other doses, the 5 mg/kg group did not significantly decrease

water drinking over time due to lower alcohol intake and compensatory water intake (data not shown).

The RMANOVA performed on food intake produced a significant effect of time, $F(5,180)=22.309$, $p<0.001$ but no effect of group, and no time by group interaction. The results for body weight were similar to those for food intake, indicating only a significant effect of time, $F(6,216)=8.785$, $p<0.001$. Post hoc tests on the main effect of time revealed a slight upward trend in body weight ($p<0.005$) in contrast to food intake, which tended to decrease over time as the rats increased their alcohol intake ($p<0.005$).

Experiment 2: High-dose paliperidone plus desipramine reduces initiation of alcohol drinking and preference in the P rat

Alcohol Intake—Two-way repeated measures ANOVA produced a significant effect of time, $F(19,684)=14.402$, $p<0.001$ and of group, $F(5,36)=12.048$, $p<0.001$ as well as a significant time by group interaction, $F(95,684)=2.435$, $p<0.001$, on alcohol intake in the rat. Animals in the 2.5 mg/kg PAL + 3 mg/kg DMI and 5 mg/kg PAL + 3 mg/kg DMI groups drank minimal or no alcohol (Figure 2A). Tukey tests demonstrated that the two drug combination groups drank significantly less alcohol than VEH consistently throughout the treatment period ($p < 0.05$). The 2.5 mg/kg PAL combination also differed from the 2.5 mg/kg PAL alone group on days 7-20, and from DMI alone on days 6, 8, 10-20 ($p < 0.05$). The 5 mg/kg combination differed from the 5 mg/kg PAL alone group on days 8-9, 12-20, and from DMI alone on days 8, 10-20. There were upward trends in alcohol intake in all single-drug treatment groups and the VEH group ($p < 0.05$).

Because of the dramatic effect of the drug combination groups on alcohol intake, we continued follow up of all animals for another 20 days after the treatment period (see 2C). Alcohol drinking in the two combination groups increased rapidly following the termination of treatments, but there was still an overall group effect on alcohol intake during the post hoc period, ($F(5,32)=2.609$, $p<0.05$).

Alcohol Preference—The results for alcohol preference in the rat were similar to those for alcohol intake. The RMANOVA analysis showed an effect of time, $F(19,684)=8.690$, $p<0.001$, a large group effect, $F(5,36)=16.348$, $p<0.001$ as well as a significant time by group interaction, $F(95,684)=3.127$, $p<0.001$. Alcohol preferences in the drug combination groups were minimal and indistinguishable from each other. Post hoc tests revealed that the drug combination groups exhibited substantially lower preference for alcohol than VEH as well as the PAL and DMI alone groups (Figure 2B). The combination of 2.5 mg/kg of PAL with DMI decreased alcohol preference compared to VEH on days 1-20, as did the combination with 5 mg/kg ($p < 0.05$). The 2.5 mg/kg PAL combination differed significantly from 2.5 mg/kg PAL alone on days 2, 7-20 while the 5 mg/kg combination differed from 5 mg/kg PAL alone on days 8-9, 11-20. Both combination groups differed from DMI alone on days 6-20. Also, during the first 5 days of treatment, the 5 mg/kg PAL group exhibited lower alcohol preference than the VEH group ($p < 0.05$). A significant upward linear trend was observed in all groups except for the combination groups ($p<0.05$).

When treatment was terminated, a significant (post hoc) effect of group on alcohol preference was obtained $F(5,32)=7.025$, $p<0.001$. Alcohol preference increased more gradually than alcohol intake in the drug combination groups during the post hoc period. Tukey pairwise comparisons showed that after treatment ended, each combination group differed significantly from VEH and its respective PAL alone group ($p < 0.01$).

Water and Food Intake and Body Weight—The RMANOVA results for water intake showed an effect of time, $F(19,684)=11.452$, $p<0.001$, no effect of group, but a significant time by group interaction, $F(95,684)=4.092$, $p<0.001$. Both the 2.5 mg/kg and 5 mg/kg combinations drank significantly more water than VEH (days 3-20), and also more than other groups during the second half of the treatment period ($p<0.05$). Water intake trended upward in the drug-combination groups and downward in the other groups ($p < 0.05$).

The RMANOVA analysis of food intake showed a significant effect of time, $F(6,216)=3.288$, $p<0.01$ and of group, $F(5,36)=4.783$, $p<0.01$ as well as a significant time by group interaction, $F(30,216)=7.003$, $p<0.001$. The higher dose of PAL (5 mg/kg) plus DMI transiently decreased food intake compared to VEH (days 1-7, $p < 0.05$), as did DMI alone (days 1-7, 19, $p < 0.05$). The 2.5 mg/kg combination did not differ significantly from VEH at any time. Similar to water intake, there were significant linear upward and downward trends in the combination and single-drug groups, respectively ($p < 0.05$).

The RMANOVA performed on body weight in the rats revealed a significant effect of time, $F(6,216)=30.025$, $p<0.001$, group $F(5,36)=3.310$, $p<0.05$, and a significant time by group interaction, $F(30,216)=8.935$, $p<0.001$. Tukey post hoc tests showed that the rats treated with higher-dose PAL (5 mg/kg) plus DMI had significantly lower weights than VEH on days 7-19. Similarly, the VEH group weighed more than the 2.5 mg/kg of PAL combination on days 10-19 ($p<0.05$). Body weight increased over time in the vehicle group (a significant linear upward trend, $p<0.05$). A significant downward trend was observed in all drug-treated groups except the 2.5 mg/kg PAL dose ($p<0.05$).

Experiment 3: Low-dose paliperidone plus desipramine does not reduce alcohol drinking and preference in the P rat

Alcohol Intake—Two-way repeated measures ANOVA showed a significant effect of time, $F(19,779)=49.386$, $p<0.001$ but no effect of group and a significant time by group interaction $F(95,779)=1.334$, $p<0.05$, on alcohol intake in the rat. There were significant upward trends in all groups ($p<0.05$, Figure 3A). The higher-dose combination of PAL (1 mg/kg) with DMI differed from PAL (1 mg/kg) on days 11, 12, 15 and 19 ($p<0.05$), but did not differ from any other group.

Alcohol Preference—The RMANOVA results for alcohol preference showed only a significant effect of time, $F(19,779)=459.182$, $p<0.001$. Post hoc analysis showed an upward (linear) trend, independent of group ($p<0.005$; Figure 3B).

Water and Food Intake and Body Weight—The RMANOVAs performed on water and body weight showed opposing trends. There was a significant effect of time, $F(19,779)=59.881$, $p<0.001$, no effect of group and a significant time by group interaction,

$F(95,779)=4.446$, $p<0.05$ on water intake. Water drinking trended downward (linearly) in each group ($p<0.05$). For body weight, there was also a significant effect of time, $F(5,205)=97.096$, $p<0.001$, but additionally a significant effect of group and no significant time by group interaction. An overall upward linear trend was estimated (post hoc) ($p < 0.05$). Pairwise comparisons among groups showed that the high-dose PAL combination differed significantly from VEH for body weight ($p < 0.05$).

The RMANOVA analysis of food intake in the rat produced a significant effect of time, $F(5,205)=16.114$, $p<0.001$, of group $F(5,41)=4.824$; $p < 0.001$, and a significant time by group interaction, $F(25,205)=2.474$, $p<0.001$. There were significant downward linear trends in the DMI alone and the two combination groups ($p<0.05$). No significant effects of treatment on food intake were seen after day 6.

Experiment 4: Paliperidone dose-response in the hamster

Alcohol Intake—Two-way repeated measures ANOVA of daily alcohol intake during the 20-day treatment period revealed a significant effect of time, $F(23,851)=13.056$, $p<0.001$, no effect of group, and a time by group interaction, $F(92,851)=2.327$, $p<0.001$. Tukey tests demonstrated that the highest PAL dose (1.0 mg/kg) decreased alcohol drinking compared to VEH on days 1-5, 8, 10 ($p<0.05$). There were no trends visible in any of the groups, although post hoc analysis showed intermittent upward changes in VEH over time ($p<0.05$; Figures 4A).

Alcohol Preference—The RMANOVA results for alcohol preference in the hamster produced a significant effect of time, $F(23,851)=13.773$, $p<0.001$, but no effect of group and no time by group interaction. The lack of a significant linear trend, coupled with a quadratic effect over time ($p < 0.005$), is consistent with the absence of strong observable trend in alcohol preference (Figure 4B).

Water and Food Intake and Body Weight—The RMANOVA performed on water intake showed that there was a significant effect of time, $F(23,851)=22.907$, $p<0.001$, no effect of group and no group by time interaction. No overall linear trend was visible in the data for water intake but a significant quadratic trend was obtained ($p<0.05$). The RMANOVA results for food intake in the hamster were similar to those for water intake, indicating only an effect of time, $F(7,259)=16.195$, $p<0.001$. Post hoc tests revealed a significant linear trend in food intake over time ($p<0.005$). For body weight, the RMANOVA analysis showed no significant effect of time or group, but a time by group interaction, $F(28,259)=2.241$, $p<0.001$ (data not shown).

Experiment 5: High-dose paliperidone plus desipramine reduces alcohol drinking and preference in the hamster

Alcohol Intake—The RMANOVA performed on alcohol intake in the hamster revealed a significant effect of time, $F(23,552)=17.645$ $p<0.001$ and of group, $F(3,24)=4.494$, $p<0.05$ and a time by group interaction, $F(69,552)=3.942$, $p<0.001$. The group treated with PAL and DMI drank significantly less than the VEH group throughout the treatment period ($p<0.05$) on days 2, 4-5, 7, 9, 10-19. The combination group also drank significantly less than PAL

alone group on days 16-19, and from DMI alone on days 15-19 (Figure 5A). The single-drug groups did not differ from VEH on any treatment day. Only the combination group displayed a significant downward trend across time ($p < 0.05$).

Alcohol Preference—The RMANOVA results for alcohol preference in the hamster were similar to those for alcohol intake, demonstrating a significant effect of time, $F(23,552)=17.364$, $p < 0.001$ group, $F(3,24)=9.920$, $p < 0.001$ and time by group interaction, $F(69,552)=5.939$, $p < 0.001$. The group treated with PAL plus DMI showed significantly less preference for alcohol than VEH for much of the treatment period (days 4, 8-20, $p < 0.05$). The combination group also showed significantly less alcohol preference compared to PAL alone and to DMI alone on days 8-20 (Figure 5B). Similar to alcohol intake, neither of the single-drug groups differed from VEH on any day and displayed no trends over the course of treatment. There was a significant downward trend in alcohol preference in the combination group ($p < 0.05$).

Water and Food Intake and Body Weight—The RMANOVA analysis of water intake in the hamsters indicated a significant effect of time, $F(23,552)=24.808$, $p < 0.001$, and of group, $F(3,24)=4.910$, $p < 0.01$, as well as a group by time interaction, $F(69,552)=3.277$, $p < 0.001$, on water intake. Tukey tests demonstrated that the group treated with PAL plus DMI drank significantly more water than VEH on days 12, 15-20, and similarly drank more water than the single-drug groups ($p < 0.05$). Increased water intake over time in the PAL plus DMI group was significant ($p < 0.05$) and coincided with decreases in alcohol intake and preference, suggesting compensatory changes in water intake in response to changes in alcohol drinking. There was also a significant downward trend in water intake in the group treated with DMI ($p < 0.05$).

The RMANOVA results for food intake in the hamster showed a significant effect of time, $F(7,168)=28.791$, $p < 0.001$ no effect of group, but a significant time by group interaction, $F(21,176)=4.111$, $p < 0.001$. The group treated with PAL plus DMI ate significantly more than controls later in the study (days 6, and 12-19, $p < 0.05$). The post hoc analysis demonstrated that food intake trended upward in the combination group, possibly to compensate for the decreases in caloric intake due to reductions in alcohol intake, and downward in the VEH group ($p < 0.05$).

The RMANOVAs on body weight revealed no effect of time, a significant effect of group, $F(3,24)=3.249$, $p < 0.05$, as well as a significant time by group interaction, $F(21,176)=3.831$, $p < 0.001$. The group treated with PAL alone weighed significantly less than VEH on days 12-19 ($p < 0.05$). There were no significant within-group trends in body weight over time.

DISCUSSION

The major finding of this study is that the atypical antipsychotic paliperidone co-administered with the norepinephrine reuptake inhibitor desipramine (3 mg/kg) essentially prevented initiation of alcohol drinking and acquisition of alcohol preference in the P rat, and almost as dramatically suppressed chronic alcohol intake and alcohol preference in the hamster. In the P rat, a line of alcohol-preferring rodent considered the benchmark animal

model of alcoholism (and which, like patients with schizophrenia, is known to have a dysregulated brain reward circuit (Engleman et al. , 2006, Morzorati, 1998)) this drug combination drastically reduced alcohol drinking to less than 1 g/kg/day. The extent of the effect of this drug combination has rarely been seen before in this line selectively bred for excessive alcohol drinking. Indeed, the potency of this drug combination for the rat appears to be far superior to that of naltrexone, a mainstay treatment for alcohol abuse, in a similar drinking paradigm in the P rat (Sable et al. , 2006). In the hamster, which we have used as a model of chronic alcohol drinking in patients with schizophrenia (Chau et al. , 2010), the reductions in alcohol drinking after treatment with paliperidone and desipramine were much greater in magnitude compared to our previous study assessing the combined effects of risperidone and desipramine (Gulick, Chau, 2014). Also noteworthy is that for both rat and hamster experiments, paliperidone and desipramine clearly acted in a synergistic manner because neither drug on its own attenuated alcohol drinking beyond (at most) modest transient effects, but together their impact was significant and substantial.

By itself, paliperidone transiently and dose-dependently attenuated initiation of alcohol intake and temporarily retarded acquisition of alcohol preference in the P rat. Paliperidone seemed more effective at suppressing chronic alcohol intake in the hamster than at suppressing the initiation of alcohol intake in the P rat (e.g., 1 mg/kg of paliperidone was able to reduce alcohol drinking in the hamster, but not in the P rat). The 5 mg/kg dose used for the P rat produced similar results (relative to vehicle controls) to that obtained using 1 mg/kg for the hamster, supporting the use of the higher dose for that animal model of alcohol drinking.

In regard to desipramine, the selected dose of 3 mg/kg did not significantly impact alcohol intake or alcohol preference in either animal model. The dose was chosen to be below those previously shown to suppress alcohol drinking in the P rat (Murphy et al. , 1985), the intent being to avoid dominant effects in the drug combination. This dose has also been shown to only have modest ability to decrease alcohol drinking in the hamster (Khokhar et al. , 2015). Prior rat studies (McBride et al. , 1988) have shown that desipramine suppresses alcohol drinking at higher doses of desipramine (5 and 10 mg/kg), although in those studies an alcohol drinking maintenance paradigm was used. In all, our result with desipramine alone is generally consistent with the rat study of Murphy et al (1985). Both our study and their study showed that the effect of desipramine can last for at least 24 hours -- desipramine suppressed alcohol drinking in the P rat by one-half of control on the first day. However, our study further shows that the P rat develops tolerance to such effect of desipramine within 4 days. This observation is consistent with the fact that chronic treatments with other norepinephrine inhibitors are usually unable to chronically suppress alcohol drinking in the P rat (Rockman et al. , 1982). Moreover, our data for both animals are consistent with human data suggesting desipramine by itself is not an overly effective anti-alcohol abuse agent (Litten and Allen, 1998).

The combination of paliperidone plus desipramine did, however, significantly decrease body weight in the P rat (relative to vehicle controls). The fact that desipramine when administered as a single drug also reduced body weight, but did not alter alcohol drinking or preference, suggests that the decreases in body weight during treatment with the

combination may not be related to decreases in alcohol drinking and may, rather, be related to the effects of desipramine itself, consistent with previous reports of desipramine-induced weight loss in rats (Nobrega and Coscina, 1987). In contrast to the rat, hamsters treated with high-dose paliperidone (2.5 mg/kg) plus desipramine ate significantly more than vehicle controls but showed no trend in body weight over time. For the two animal experiments, there was no systematic pattern to changes in food intake and body weight that would support the general supposition that high doses of the drugs either singly or in combination may reduce general consummatory behavior. Lastly, since neither desipramine, nor paliperidone, have been reported to alter the metabolism or clearance of alcohol (Monostory et al. , 2004, Simon O'Brien et al. , 2011) the decrease in alcohol drinking is probably not due to altered alcohol pharmacokinetics.

We note two limitations in our study. First, while we studied the effects of paliperidone with and without desipramine on the initiation of alcohol drinking in the P rat, we did not study the possible effect of these agents on chronic alcohol drinking in this animal. Our choice to not study chronic alcohol drinking was based on our previous work demonstrating that clozapine decreases the initiation of alcohol drinking in this animal, but does not significantly decrease its chronic alcohol drinking. While we believe that initiation of alcohol drinking in the P rat more closely models alcohol drinking in patients with schizophrenia (as noted above), in subsequent studies we intend to assess the effects of these agents on chronic drinking in the P rat. Second, while we studied the effect of pharmacologic agents on alcohol drinking in the P rat and in the hamster, we did not also study their effect on consumption of sucrose, known to also be preferred by these animals. Although in previous studies, we reported that clozapine's effect on alcohol drinking in the hamster was not paralleled by a similar effect on sucrose drinking (Chau, Gulick, 2010), we also intend to assess new agents on sucrose drinking as well.

In summary, the current data confirm our hypothesis that paliperidone itself does not chronically attenuate alcohol drinking, but that it is able to do so when co-administered with the norepinephrine reuptake inhibitor desipramine. On the basis of the current data, we speculate that patients with schizophrenia with comorbid alcohol use disorder may benefit from use of paliperidone in combination with desipramine, a medication known to block the reuptake of norepinephrine. Finally, in light of the known pharmacological properties of paliperidone and desipramine, as described in the introduction, the current data suggest that this drug combination, which exhibits norepinephrine reuptake inhibition, potent blockade of norepinephrine alpha-2 receptors, and relatively modest blockade of dopamine D2 receptors may be useful (and should be tested) in the treatment of alcohol use disorder in patients with schizophrenia, and potentially those with alcohol use disorder alone.

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HIGHLIGHTS

- Paliperidone alone reduces alcohol drinking in P rat (initiation) and hamster
- Paliperidone and desipramine abolish initiation of alcohol drinking in P rat
- Paliperidone and desipramine reduce chronic alcohol drinking in the hamster

Figure 1.

Paliperidone, at high doses, transiently reduces initiation of alcohol drinking and preference in the P-rat. (A) The highest PAL dose (5 mg/kg) delayed the initiation of alcohol (A) intake (post hoc differences from VEH are indicated by asterisks by group and day) and (B) preference (pairwise differences from VEH are indicated in the legend with an asterisk) compared to vehicle control (N=6-7/group; * $p < 0.05$).

Figure 2.

Paliperidone (high doses), combined with desipramine, significantly reduces initiation of alcohol drinking in the P-rat. PAL plus DMI completely blocked the initiation of (A) alcohol drinking and decreased (B) alcohol preference compared to controls throughout the experiment. (C) Upon termination of drug treatment, alcohol intake quickly returned toward levels seen in the vehicle treated group ($N=7/\text{group}$; $*p<0.05$ post hoc differences from VEH are indicated by asterisks by group and day).

Figure 3. Low-dose PAL plus DMI does not affect initiation of alcohol drinking in the P-rat. (A) There were no significant effects on (A) alcohol intake or (B) preference in animals receiving lower dose combinations of PAL (0.5 or 1 mg/kg) and DMI. (N=6-7/group).

Figure 4.

Paliperidone transiently decreases alcohol drinking in the hamster. PAL decreased (A) alcohol drinking but not (B) alcohol preference initially and this effect diminished over time. (Day 0 indicates start of treatment; N=6-7/group; * $p < 0.05$ post hoc differences from VEH are indicated by asterisks by group and day).

Figure 5.

Paliperidone, combined with desipramine, significantly reduces alcohol drinking in the hamster. PAL (2.5 mg/kg) plus DMI (3 mg/kg) significantly reduced (A) alcohol intake and (B) alcohol preference. (day 0 indicates start of treatment; N=6-7/group; * $p < 0.05$ post hoc differences from VEH are indicated by asterisks by group and day)