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The Effects of the Preferential Dopamine D₃ Receptor Antagonist S33138 on Ethanol Binge-Drinking in C57BL/6J Mice

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Abstract

The mesolimbic dopamine (DA) pathway plays an integral role in the reinforcing properties of many drugs of abuse, including alcohol (ethanol/EtOH). It has been reported that selective and acute blockade of the DA D₃ receptor by SB-277011A will attenuate EtOH preference, intake, and lick responses in EtOH preferring rats. However, alcohol consumption that leads to abuse is often marked by binge drinking - which is characterized as bringing ones blood EtOH levels to 80mg/dl within two hours of the initial drink. It is unclear if brain mechanisms implicated in EtOH reward are equally implicated in EtOH binge consumption and abuse. Therefore, in this study, we examined the effect of the preferential D₃ receptor antagonist S33138 on ethanol (6% v/v) and water consumption in male C57BL/6J mice using a restricted-access binge-drinking model. Ethanol drinking was not significantly altered by the i.p. administration of 0.16 mg/kg of S33138. In contrast, the i.p. administration of 0.64 or 2.5 mg/kg i.p. of S33138 produced a significant decrease in ethanol consumption on days 1 and 7 and days 7-14 compared to vehicle treated animals. However, the mean water consumption was significantly decreased by 1) 0.16 and 0.64 mg/kg i.p. of S33138 on Day 1 and 2) 2.5 mg/kg i.p. of S33138 at Days 1, 7 and 7-14. Our studies indicate that a low dose of S33138 significantly decreases binge drinking, and that it does not significantly alter the consumption of water. In addition, S33138 alone is not appetitive.

Keywords

dopamine; D₃ receptor; binge drinking; ethanol

INTRODUCTION

Alcohol is one of the most widely used and abused drugs in our society. A recent survey showed that over half of Americans 12 years old and above admitted to being a current user of alcohol, and 23.7% of the same group acknowledged binge-drinking (five or more drinks within a couple hours) within the last 30 days (SAMHSA, 2010). There is considerable evidence indicating that dopamine plays a role in mediating the rewarding and reinforcing actions of natural rewards and drugs of addiction (for reviews Gardner, 2011 and Wise, 2008). In addition, numerous studies suggest that D₃ receptors are involved in mediating the appetitive and reinforcing actions of drugs of addiction (Spiller et al., 2008; Song et al., 2011). Recent studies have reported the systemic administration of the selective D₃ antagonists SB-277011-A significantly decreases ethanol seeking and relapse in mice (Heidbreder et al., 2007) and rats (Vengeliene et al., 2006) and significantly decreases ethanol preference and intake in alcohol-preferring rats (Thanos et al., 2005). However, to date, there have been no reports of the effects of a D₃ receptor antagonist in an animal model of ethanol binge drinking. This is important as binge drinking is a strong predictor of alcohol

dependence (WHO, 2009). The National Institute on Alcohol Abuse and Alcoholism (NIAA) defines binge drinking as bringing one's blood alcohol level to 80mg/dl within two hours of the initial drink. In this animal model of binge drinking, mice are limited to drinking [EtOH] for two hours and their blood alcohol levels are determined two hours after their initial drink. Therefore, in this study, we examined the effect of the systemic administration of S33138-1, a preferential D₃ antagonist (Millan et al. 2008a), on the intake of ethanol in a binge drinking model in mice.

MATERIALS AND METHODS

Mice were treated in accordance with the United States Public Health Service Guide for the Care and Use of Laboratory Animals, and all protocols were approved by Furman University's Institutional Animal Care and Use Committee. Seventy-eight twelve-week old male C57 mice were purchased from The Jackson Laboratory (Bar Harbor, Maine.) Animals were individually housed in a controlled environment and subjected to a 12 h/12 h reverse light/dark cycle (lights off at 0700 hours.) Mice were given 24 hour access to food and water for five days to allow them to acclimate to their environment. Mice's weights and overall health was monitored daily. Animals were normalized to a restricted-access binge-drinking paradigm by first limiting daily water access to 8 hours (0900 hours – 1700 hours) for 3-5 days, then two hours daily (0900 hours – 1100 hours) for 3-5 days. After 2-hour water drinking levels were consistent, animals in the ethanol portion of the study were given access to de-carbonated nonalcoholic beer (Coors® NA, de-carbonated using a Nalgene® vacuum apparatus) for 2 hours daily (0900 hours – 1100 hours) until consistent drinking was maintained. Animals in the water portion were maintained on 2-hour water consumption during this period. Following this normalization to binge-drinking, de-carbonated nonalcoholic beer was spiked to form a 6% v/v ethanol solution. Animals in the ethanol portion of the study were randomly assigned to one of three S33138 doses; 0.16 mg/kg (n=10), 0.64 mg/kg (n=9), 2.5 mg/kg S33138 (n=10) or a vehicle (de-ionized water) group (n=10). Subsequently, mice were given access to ethanol in the 2-hour binge-drinking session previously mentioned for three consecutive days (denoted N) to establish baseline ethanol consumption. Animals in the water portion of the study were randomly assigned to one of four S33138 doses; 0.16 mg/kg (n=10), 0.64 mg/kg (n=9), 2.5 mg/kg (n=10) or 5.0 mg/kg S33138 (n=10). On the fourth day (denoted T1), each animal was injected intraperitoneally (IP) with its assigned S33138 dose 30 minutes prior to drinking, for 14 consecutive days. S33138 (Servier, France) was added to de-ionized water and mixed using a vortex until completely dissolved. 0.16, 0.64, 2.5 and 5.0 mg/kg doses were prepared fresh daily (a 5.0 mg/kg dose was not used in the ethanol portion of the study). We compared drinking between the groups and within groups. A One-way ANOVA with the significance level set at $p < 0.05$ and a Tukey's post-hoc analysis was used in this study.

RESULTS

S33138, administered at the 0.64 mg/kg and 2.5 mg/kg doses, decreased ethanol consumption on days T1, T7, and T7-14 (Figure 1.) One-way ANOVA revealed significant differences in ethanol consumption between doses for days T1 ($F=7.431$; $df=3, 35$; $p=0.001$), T7 ($F=8.942$; $df=3, 35$; $p<0.001$), and T7-14 ($F=17.819$; $df=3, 308$; $p<0.001$.) The Tukey post hoc test showed that animals exposed to 0.64 mg/kg S33138 consumed significantly less ethanol than those receiving vehicle treatment on T1 ($p=0.001$), T7 ($p=0.001$), and T7-14 ($p<0.001$). Animals treated with 2.5 mg/kg S33138 consumed significantly less ethanol than vehicle animals on T1 ($p < 0.05$), T7 ($p=0.001$), and T7-14 ($p<0.001$). There were no significant differences in ethanol consumption between doses for N ($F=0.702$; $df=3, 35$; $p=0.557$), T3 ($F=1.009$; $df=3, 35$; $p=.400$), or T14 ($F=1.395$; $df=3, 35$; $p=0.261$.) Additionally, a One-way ANOVA revealed no significant differences in water

consumption between groups for N ($F=0.635$; $df=3, 35$; $p=0.598$). A one-way repeated measures ANOVA revealed significant within-group differences in water consumption when compared to the respective group's baseline consumption for the 0.16 mg/kg group ($F=10.922$; $df=4, 45$; $p<0.001$), the 0.64 mg/kg group ($F=4.016$; $df=4, 39$; $p=0.008$) the 2.5 mg/kg group ($F=20.382$; $df=4, 43$; $p<0.001$) and the 5.0 mg/kg group ($F=51.097$; $df=4, 44$; $p<0.001$). The Tukey posthoc test showed that 0.16 mg/kg S33138 decreased water consumption on T1 ($p=0.001$) but no other time of interest; the 0.64 mg/kg dose decreased water consumption on T1 ($p=0.014$) but not other time of interest; the 2.5 mg/kg dose decreased water consumption on T1 ($p<0.001$), T7 ($p<0.001$), T14 ($p=0.001$) but not T3 ($p=0.204$); and the 5.0 mg/kg dose decreased water consumption on all days ($p<0.001$).

DISCUSSION

Our study indicates that the preferential dopamine D_3 vs. D_2 receptor antagonist, S33138, significantly decreases the self-administration of ethanol in a restricted-access binge-drinking paradigm in mice. This is the first study to report such a finding. S33138, at the 0.64 mg/kg and 2.5 mg/kg doses, significantly decreased ethanol consumption on days 1, 7, and 7-14. The fact that there were no significant differences in drinking on days 3 and 14 can likely be attributed to the sporadic daily drinking during the first few days of S33138 administration or to unavoidable external factors such as noise or other stressors. Our findings are congruent with other studies that have examined the effect of D_3 antagonists on the appetitive effects of ethanol using non-binge drinking or intake models in rodents. For example, Thanos et al. (2005) reported that the selective D_3 receptor antagonist SB277011-A significantly attenuated ethanol preference, lick responses, and intake in both ethanol preferring and non-preferring rats. In addition, the pretreatment with the D_3 antagonist SB277011-A significantly attenuated ethanol seeking and significantly decreased the relapse to ethanol consumption in rats (Vengeliene et al., 2006). Similarly, Heidbreder et al. (2007) showed that, in mice, SB277011-A (30 mg/kg) significantly reduced alcohol intake and significantly reduced the reinstatement of alcohol seeking in a dose dependent manner.

There was a decrease in water consumption when compare to baseline drinking for all doses (0.16 mg/kg – 5.0 mg/kg) of S33138 on the first day of drug administration. This result is likely a consequence of receiving an injection for the first time as the animals showed signs of stress after being injected and exhibited a decrease in locomotion (unpublished observations). This explanation is supported by the data for T3, in which the drinking levels for all groups had rebounded. Animals treated with 0.16 or 0.64 mg/kg i.p. of S33138 for 14 days showed no significant changes in water consumption from baseline levels, suggesting that blocking the D_3 receptor does not decrease the motivation for water. However, animals exposed to 2.5 or 5 mg/kg of S33138 (2.5 and 5.0 mg/kg) consumed significantly less water than during the baseline period. These effects could be mediated by a blockade of D_2 rather than D_3 receptors, as the D_3/D_2 ratio is about 25-fold for D_3 vs. D_2 receptors (Millan et al., 2008a). Thus, it is possible that once a certain dose of S33138 has been reached, it activates D_2 receptors and subsequently affects drinking behavior. This is congruent with data indicating that the acute administration of 2.5 or 5 mg/kg of S33138 produced a significant inhibition of sucrose self-administration and rotarod performance in rats. (Peng et al., 2009). In addition, the chronic (21 days) i.p. administration of 10 mg/kg i.p. of S33138, but not 0.16, 0.64 or 2.5 mg/kg, produced a decrease in the number of spontaneously active A9 and A10 DA neurons, an effect likely due to the antagonism of D_2 receptors (Millan et al., 2008b). It is unlikely that the decrease in ethanol consumption produced by 0.16 and 0.64 mg/kg of S33138 is due to psychomotor impairment as these doses do not significantly alter locomotor activity or rotarod performance (Peng et al., 2009). Finally, it is unlikely that S33138 is a substitute for ethanol as alone, selective D_3 antagonists in rodents are not self-

administered, do not produce a significant left shift in brain stimulation reward and do not produce a conditioned place preference response (Heidbreder and Newman, 2010)

In conclusion, the results of this study indicated that S33138 significantly attenuates the consumption of ethanol in a mouse model of binge drinking. It is likely that at doses below 2.5 mg/kg, this effect is due to the antagonism of D₃ receptors. Our results are congruent with studies reporting that antagonism of D₃ receptors attenuates the oral intake of ethanol in rodents. If these results can be translated to humans, they suggest that D₃ receptor antagonists may be useful in the pharmacological treatment of binge drinking.

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Mean Ethanol Consumption

Figure 1.

Mean ethanol consumption in daily 2-hour binge-drinking session over 14 days. Each data point represents the mean consumption of ethanol S.E.M. * Indicates a significant difference in ethanol consumption from the 0 mg/kg group at $p < 0.05$ for the specific day of interest, and *** indicates a significant difference at $p = 0.001$.

Figure 2.

Mean water consumption in daily 2-hour binge-drinking session over 14 days. There were no significant differences in water consumption between S33138 dose groups during the baseline (N) period ($p=0.598$.) * Indicates a significant difference in water consumption from normalization drinking at $p<0.05$. ** Indicates a significant difference at $p<0.01$, and *** indicates a significant difference at $p<0.001$.