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The α_2-adrenergic receptor agonist, clonidine, reduces alcohol drinking in alcohol-preferring (P) rats

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A B S T R A C T

Evidence suggests that noradrenergic signaling may play a role in mediating alcohol-drinking behavior in both rodents and humans. We have investigated this possibility by administering clonidine to alcohol-drinking rats selectively bred for alcohol preference (P line). Clonidine is an α_2-adrenergic receptor agonist which, at low doses, inhibits noradrenergic signaling by decreasing norepinephrine release from presynaptic noradrenergic neurons. Adult male P rats were given 24 h access to food and water and scheduled access to a 15% (v/v) alcohol solution for 2 h daily. Rats received intra-peritoneal (IP) injections with clonidine (0, 10, 20, 40, or 80 μg/kg body weight [BW], 10–11 rats/treatment group) once/day at 30 min prior to onset of the daily 2 h alcohol access period for 2 consecutive days. Clonidine, in doses of 40 or 80 μg/kg BW, significantly reduced alcohol intake on both days of treatment ($p < 0.001$). Two weeks later, rats were treated with clonidine for 5 consecutive days and clonidine, in doses of 40 or 80 μg/kg BW, reduced alcohol intake on all 5 treatment days ($p < 0.001$). Clonidine did not alter water consumption during the daily 2 h free-choice between alcohol and water. In a separate group of male P rats, clonidine (40 μg/kg BW) suppressed intake of a saccharin solution (0.04 g/L). These results are consistent with and complement our previous findings that the α_1-adrenergic receptor antagonist, prazosin, decreases voluntary alcohol drinking in alcohol-preferring rats, but suggests that effects of clonidine may not be specific for alcohol. The results suggest that although activation of the noradrenergic system plays an important role in mediating voluntary alcohol drinking, care is needed in selecting which drugs to use to suppress central noradrenergic signaling in order to maximize the selectivity of the drugs for treating alcohol-use disorders.

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Introduction

It has been suggested that excessive noradrenergic activation which accompanies anxiety and hyperarousal may contribute to increased alcohol drinking in an effort to self-medicate, since alcohol is sympatho-suppressive, anxiolytic, and sedating (Edwards, Chandler, Hensman, & Peto, 1972; Koob & LeMoal, 1997; Kushner, Sher, & Beitman, 1990; Kushner, Sher, & Erickson, 1999; Shirao et al., 1988). Numerous lines of evidence support this view: a) anxiety is associated with increased brain noradrenergic and associated sympathoadrenal activation (Kopin, 1984; Sullivan, Coplan, Kent, & Gorman, 1999), b) "anxious" rats consume more alcohol than "non-anxious" rats (Spanagel et al., 1995), c) blocking norepinephrine biosynthesis decreases alcohol self-administration by rodents (Amit, Brown, Levitan, & Ogren, 1977; Brown, Amit, Levitan, Ogren, & Sutherland, 1977; Davis, Smith, & Werner, 1978), d) alcoholics commonly state that relief of anxiety is an important reason for drinking (e.g., Edwards et al., 1972), e) alcoholism co-occurs at high rates with anxiety disorders (Kushner et al., 1990), suggesting that the two disorders represent manifestations of similar underlying mechanisms (Merikangas, Risch, & Weissman, 1994; Merikangas et al., 1998; Sinha, Robinson, & O'Malley, 1998), f) patients with co-morbid anxiety and alcoholism more frequently report that they use alcohol to control anxiety and panic symptoms as compared to other reasons for alcohol use (Kushner et al., 1990), g) increased sympathetic activation is seen during periods of increased anxiety and during prolonged alcohol abstinence (Ehrenreich et al., 1997; Sullivan et al., 1999), and h) increased sympathoadrenal activation and anxiety-like behavior is observed for long periods following termination of chronic alcohol consumption in rats (Rasmussen, Mitton, Green, & Puchalski, 2001; Rasmussen, Wilkinson, & Raskind, 2006). Taken together, these

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findings suggest that excessive sympathetic activation may contribute not only to maintenance of alcohol drinking and alcohol abuse but may also be one of the aversive physiological events that occur during alcohol withdrawal and abstinence that increases risk of relapse to alcohol drinking (Koob & LeMoal, 1997).

We previously tested the hypothesis that noradrenergic activation promotes and maintains alcohol drinking by assessing whether alcohol drinking in rats is decreased by prazosin treatment. Prazosin is a drug that is centrally active when administered peripherally and that decreases brain noradrenergic signaling by blocking postsynaptic α_1-adrenergic receptors. Prazosin dose-dependently reduced withdrawal-induced operant self-administration of alcohol in alcohol-dependent Wistar rats (Walker, Rasmussen, Raskind, & Koob, 2008). Prazosin also suppressed voluntary alcohol drinking by rats selectively bred for alcohol preference (P line) when administered either acutely (Rasmussen, Alexander, Raskind, & Froehlich, 2009) or chronically (Froehlich, Hausauer, Federoff, Fischer, & Rasmussen, 2013; Froehlich, Hausauer, & Rasmussen, 2013). The ability of prazosin to reduce alcohol drinking has been confirmed in humans; Simpson et al. (2009) reported that prazosin decreased relapse alcohol drinking in treatment-seeking alcohol-dependent men. These results from both rodents and humans provide compelling evidence that noradrenergic activation plays an important role in mediating alcohol drinking and alcohol relapse.

If alcohol drinking is due in part to activation of the noradrenergic system, any drug that decreases noradrenergic signaling might be expected to decrease alcohol drinking. We recently determined that combined treatment of P rats with both prazosin and propranolol (which block α_1- and β-adrenergic receptors, respectively) decreased alcohol drinking more effectively than treatment with either drug alone (Rasmussen, Beckwith, Kincaid, & Froehlich, 2014), suggesting that α_1- and β-adrenergic receptors may have complementary roles in facilitating alcohol drinking. This suggests that administration of an α_2-adrenergic receptor agonist such as clonidine, which can decrease noradrenergic signaling by decreasing norepinephrine release from presynaptic terminals (Aghajanian & VanderMaelen, 1982; Starke, Montel, Gayk, & Merder, 1974) and thus decrease the amount of norepinephrine available for binding to either α_1- or β-adrenergic post-synaptic receptors, may be especially effective in decreasing alcohol drinking. Accordingly, in the present study we evaluated the effect of clonidine on voluntary alcohol drinking in selectively bred alcohol-preferring (P) rats.

Materials and methods

Animals

Male P rats ($n = 51$ in Study 1; $n = 20$ in Study 2) from the 60th generation of selective breeding for alcohol preference served as subjects. The rats were individually housed in stainless-steel hanging cages in an isolated vivarium with controlled temperature (21 ± 1 °C) and a 12 h light/dark cycle (lights off at 1000 h). Standard rodent chow (Laboratory Rodent Diet #7001, Harlan Teklad, Madison, WI) and water were available *ad libitum* at all times throughout the study. All experimental procedures were approved by the Indiana University Institutional Animal Care and Use Committee and conducted in strict compliance with the NIH Guide for the Care and Use of Laboratory Animals.

Six months prior to onset of the current study, the rats in Study 1 were treated acutely with intra-peritoneal (IP) prazosin for 2 consecutive days and then, 3 weeks later, for 5 consecutive days (Rasmussen et al., 2009). The rats were then held for 6 months without drug treatment prior to onset of the current clonidine study. During this time, all rats received 2 h access to alcohol (15% v/v) for 2 h/day, 5 days/week until the current investigation. The rats were 13 months old with a mean body weight of 679 g at the start of clonidine treatment.

Drugs

Clonidine hydrochloride (Sigma–Aldrich Co., St. Louis, MO) was dissolved in 0.9% NaCl. In Study 1, each rat received an IP injection of saline vehicle or 10, 20, 40, or 80 μg clonidine/mL saline/kg BW. In Study 2, each rat received an IP injection of saline vehicle or 40 μg clonidine/mL saline/kg BW.

Study 1: effects of clonidine treatment on alcohol intake

The alcohol solution was prepared by diluting 95% alcohol (ethanol; Decon Laboratories Inc., King of Prussia, PA) with distilled, deionized water to make a 15% (v/v) solution. Alcohol (15% v/v) and water were presented in calibrated glass drinking tubes, with positions of the tubes alternated daily to control for potential side preferences. Daily fluid intakes were recorded to the nearest mL. Alcohol intake was converted from mL alcohol/kg BW to g alcohol/kg BW.

During the week prior to clonidine administration, baseline alcohol and water intakes during the daily 2 h alcohol-access period (1100–1300 h) were calculated for each rat over 4 consecutive days and the rats were ranked in descending order in terms of average daily alcohol consumption. The rats were assigned to drug treatment groups in a manner that ensured that the groups did not differ in baseline alcohol intake prior to clonidine administration. Specifically, the top 5 alcohol drinkers were randomly assigned to the vehicle or one of four clonidine-treatment groups (10, 20, 40, or 80 μg clonidine/kg BW), followed by the next 5 highest alcohol drinkers likewise randomly assigned, etc.

2-day clonidine treatment

To reduce stress associated with IP drug administration, all rats were handled as if they were going to receive an IP injection for 5 consecutive days prior to onset of drug treatment, and all rats received an IP injection of vehicle on the day preceding onset of drug treatment. Clonidine in doses of 10, 20, 40, or 80 μg/kg BW ($n = 10$–11/dose) or an equivalent volume of vehicle ($n = 10$–11) was administered 30 min prior to the daily 2 h alcohol-access period (1100–1300 h, beginning 1 h after lights out) on each of 2 consecutive days.

5-day clonidine treatment

After completion of the 2-day clonidine treatment regimen, rats continued to receive 2 h (1100–1300 h) daily access to alcohol 5 days/week for 3 weeks prior to initiating 5 days of clonidine treatment. To reduce the stress associated with IP drug administration, all rats were again handled as if they were going to receive an IP injection for 5 days prior to treatment and were given an IP injection of vehicle on the day preceding onset of 5 days of drug treatment. Average daily alcohol and water intakes were determined in the week preceding drug treatment and the rats were rank ordered, based on alcohol intake, and re-assigned to treatment groups as previously described. Clonidine, in doses of 10, 20, 40, or 80 μg/kg BW, or an equivalent volume of vehicle, was administered 30 min prior to the daily 2 h alcohol access period on each of 5 consecutive days.