

Research Article

## Effects of Anabasine, a Tobacco Smoke Constituent, on Alcohol Consumption in Female Alcohol Preferring (P) Rats

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**Abstract** In addition to nicotine, there are a variety of other psychoactive compounds in tobacco smoke. Little is known about the interaction of these tobacco constituents on alcohol intake. In this study, we examined one of these constituents, anabasine, which acts on nicotinic receptors, for its effects on alcohol intake and preference in alcohol preferring (P) rats. Adult female P rats were allowed continuous 3-bottle access to drink 0%, 10% or 15% alcohol for over 6 weeks. After establishment of a stable baseline for alcohol intake, rats were injected acutely with 0.02, 0.2 or 2.0 mg/kg anabasine or saline. Alcohol and water intake were measured at 2, 4, 6, and 24 h after each treatment. Pretreatment with anabasine at 2 mg/kg significantly reduced alcohol intake during the 0–2 h and decreased alcohol preference during 2–4 h and 6–24 h periods but increased water intake during the 2–4 h period. Other doses of anabasine did not exert significant effects on alcohol or water intake. These results suggest that anabasine may influence alcohol intake presumably by interacting with neuronal nicotinic receptors.

**Keywords** nicotine; anabasine; drinking; P rats; smoking

### 1. Introduction

Tobacco and alcohol addiction are quite convergent such that approximately 90% of alcoholics are also tobacco smokers [21]. Because alcohol and tobacco are often coabused it is vital to understand their interactions to devise more effective methods to overcome both of these addictions. Nicotine is the principal psychoactive component in tobacco and research has shown that nicotine can potentiate alcohol consumption [3,24,25,26,29]. However, nicotine is not the only psychoactive compound in tobacco smoke. Other biologically active components in tobacco also play an important role in smoking and drinking [19]. There is already some evidence that some tobacco constituents have addictive liability [8]. For example, acetaldehyde, a constituent found in tobacco smoke, has been shown to have abuse liability. Animals will self-administer acetaldehyde directly into the cerebral ventricles [5] and into the ventral tegmental area (VTA) [35]. Microinjection of acetaldehyde into the posterior VTA has been shown to significantly increase dopamine levels in the nucleus accumbens of rats [12]. The  $\beta$ -carbolines

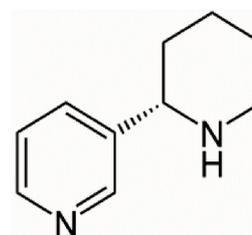


Figure 1: Molecular structure of anabasine.

harmine and norharmine found in tobacco smoke have been implicated in drinking [1] by exerting their effects on the dopaminergic system in the brain [2]. Another compound found in tobacco smoke, anabasine, has been shown to have abuse potential [8]. Anabasine, with a similar chemical structure to nicotine (see Figure 1), is a selective nicotinic acetylcholine receptor partial agonist found in tobacco leaf, green tomatoes, potatoes, and red peppers [6,20]. Anabasine acts as a putative  $\alpha 4\beta 2$  partial agonist [30,39]. In vitro studies have shown that anabasine increases midbrain levels of dopamine [15,16] which plays an important role in addiction to alcohol [14,23,27]. Anabasine has also been shown to fully substitute for nicotine [4] and partially substitute for *d*-methamphetamine in drug discrimination paradigms [13]. Furthermore, recently it was found that anabasine pretreatment reduces nicotine self-administration in mice [6] and rats [17].

Several neuronal systems including the neuronal nicotinic receptors have been implicated in alcohol drinking [9,28,31,34]. Because of similarity of anabasine with nicotine structure and properties, it was hypothesized that similar to nicotine itself anabasine may influence alcohol drinking. The main goal of this study was to assess the effects of acute systemic administration of anabasine on alcohol intake and preference at different time points in selectively-bred female alcohol preferring (P) rats.

## 2. Method

### 2.1. Subjects

Sixteen adult female selectively-bred alcohol preferring (P) rats obtained from Indiana University were used. Animals were maintained in a standard vivarium with controlled temperature of  $22 \pm 1$  °C and humidity of  $50 \pm 10\%$  under reversed 12:12 light-dark cycle (lights out at 7:00 AM). The rats were housed in single cages that were fitted with three 100 mL graduated Richter drinking tubes. One tube contained 10% alcohol, one contained 15% alcohol, and another had tap water. Animals had free access to alcohol and water and were fed Rodent Diet 5001 (Lab Diet, St. Louis, MO, USA) ad libitum. Experimental procedures were approved by the Institutional Animal Care & Use Committee (IACUC) of Duke University.

### 2.2. Initiation of drinking

One week after arrival to the lab, rats were first given access to water in Richter drinking tubes for a few days and then were exposed to alcohol solutions as the sole source of fluid for 3 consecutive days. During this forced exposure to alcohol, the rats experienced both the taste of alcohol and the reinforcing properties of alcohol [32,33]. Thereafter, they were given free access to water in one tube and 10% and 15% (v/v) solutions of alcohol in other tubes for at least 6 consecutive weeks.

### 2.3. Drug preparation

Solutions of anabasine (Sigma, Milwaukee, WI, USA) were prepared in pyrogen-free glassware in sterilized isotonic saline. Subcutaneous (SC) injections were made in a counterbalanced order 15 min before testing for alcohol self-administration. Injections were given in a volume of 1 mL/kg. For oral alcohol administration, 10% and 15% (v/v) alcohol solutions were prepared twice a week from 100% reagent grade ethyl alcohol and tap water.

### 2.4. Experiments

The main purpose of this study was to determine the effects of a biologically active constituent of tobacco, anabasine, on alcohol intake and preference. After the establishment of a reliable baseline of alcohol intake, rats were injected SC with one of the three doses of anabasine (0.02, 0.2, and 2 mg/kg) or the control saline solution; alcohol and water intake were measured at 2, 4, 6, and 24 h after each treatment. All animals received all doses of anabasine and the control vehicle following a cross-over design with random assignment. The interval between injections was at least 3 consecutive days or until the re-establishment of baseline alcohol intake.

### 2.5. Statistical analysis

The alcohol self-administration data were assessed by analysis of variance. A repeated measures design was used with anabasine dose and time interval after anabasine administration as independent factors and alcohol intake (g/kg/h), water intake (mL/kg/h), and alcohol percent preference as dependent measures. An alpha level of  $P < .05$  was used as a cutoff for statistical significance. Dunnett's tests (two-tailed) were used for post-hoc comparisons of the control versus the effect of each anabasine dose.

## 3. Results

### 3.1. Alcohol consumption

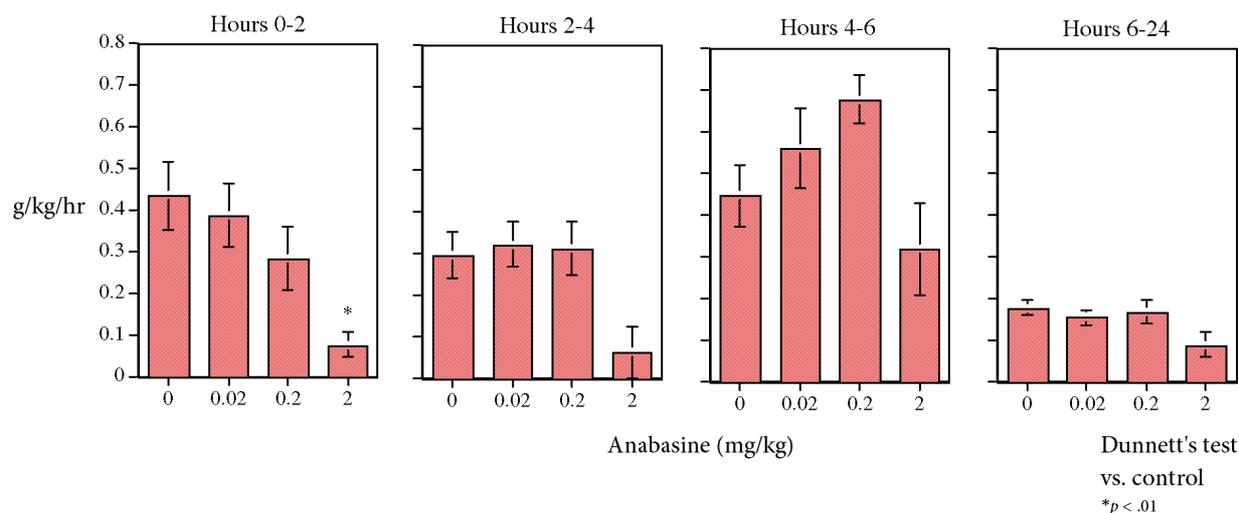
There was a significant ( $F(3,33) = 43.78, P < .0005$ ) main effect of anabasine on alcohol consumption (g/kg/h). Comparisons of each of the anabasine doses to control showed that averaged over all of the time periods, the 2 mg/kg anabasine dose significantly ( $F(1,33) = 25.22, P < .01$ ) reduced alcohol consumption relative to control. Figure 2 shows the anabasine effects on alcohol consumption over the different time periods after acute administration. Comparisons of anabasine at 2 mg/kg dose to control for each of the time periods showed significant reductions in alcohol consumption for the 0–2 h period ( $P < .01$ ). There was a nearly significant anabasine-induced reduction of alcohol consumption during the 2–4 h period, but nothing thereafter.

### 3.2. Water consumption

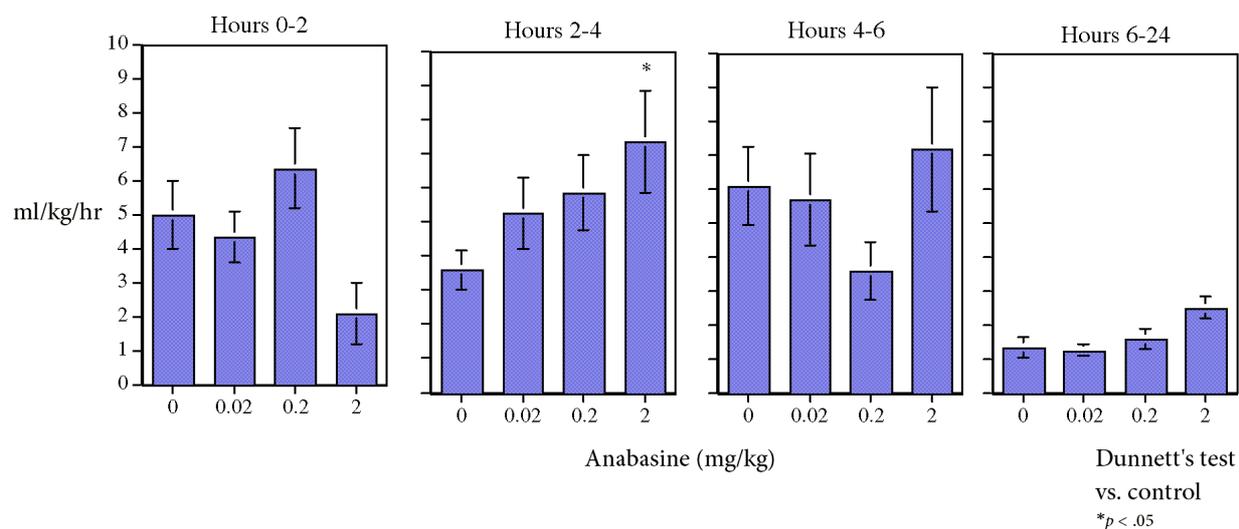
The main effect of anabasine on water consumption was not significant but the interaction of anabasine  $\times$  time period was significant ( $F(9,99) = 2.91, P < .01$ ). The anabasine effects on water consumption over time are shown in Figure 3. Comparisons of each anabasine dose to control for each time period showed that the 2 mg/kg anabasine dose caused a significant ( $P < .05$ ) elevation in water consumption relative to control during the 2–4 h period. None of the other comparisons detected significant effects.

### 3.3. Alcohol preference

There was a significant ( $F(3,33) = 11.32, P < .0005$ ) main effect of anabasine treatment with percent alcohol preference. Comparisons of each of the anabasine doses to control showed that averaged over all of the time periods the 2 mg/kg anabasine dose significantly ( $F(1,33) = 24.31, P < .01$ ) reduced alcohol preference relative to control. Figure 4 shows the anabasine effects on alcohol preference over the different time periods after acute administration. The 2 mg/kg anabasine dose caused a significant ( $P < .05$ ) reduction in alcohol preference relative to the control condition during all time periods except during 4–6 h block. The lower anabasine doses did not cause significant changes in alcohol preference.



**Figure 2:** Anabasine effects on alcohol consumption (g/kg/h). Animals were pretreated with three doses of anabasine (0.02, 0.2, and 2 mg/kg, SC) or saline and their alcohol intake was measured at 2, 4, 6, and 24 h after the treatment. Data represent mean  $\pm$ SEM.  $N = 16$ .



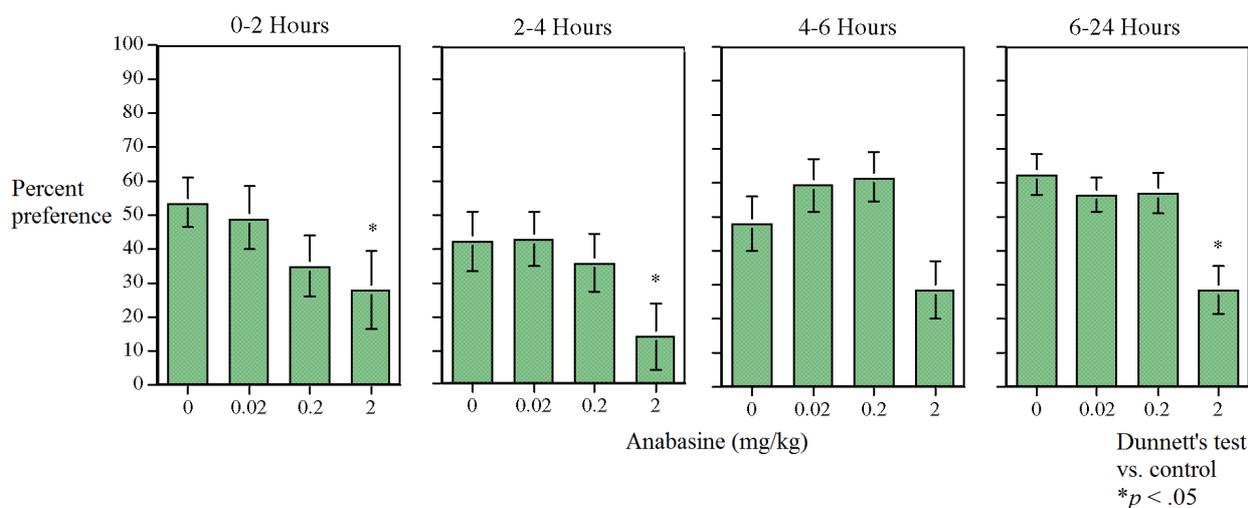
**Figure 3:** Anabasine effects on water consumption (mL/kg/h). Animals were pretreated with three doses of anabasine (0.02, 0.2, and 2 mg/kg, SC) or saline and their water intake was measured at 2, 4, 6, and 24 h after the treatment. Data represent mean  $\pm$ SEM.  $N = 16$ .

#### 4. Discussion

Minor nicotinic alkaloids present in tobacco smoke, although less potent than nicotine itself, have been shown to be pharmacologically active [19,41]. Some of these nicotinic alkaloids, which are structurally similar to nicotine, may play a role in addiction to nicotine or other drugs usually used with nicotine, such as alcohol. In the present study, we investigated the acute effect of one of these alkaloids, anabasine, on alcohol intake and preference in alcohol preferring (P) rats. Our results showed that anabasine at 2 mg/kg when given acutely can significantly reduce total alcohol intake for up to 2 h after the treatment. There was also a trend for anabasine-induced reduction in

alcohol intake during 2–4 h and 4–6 h periods after acute treatment, but it was not quite significant. The most dramatic effect of anabasine at 2 mg/kg reducing alcohol intake was seen during 0–2 h time block. Water intake was slightly reduced during the 0–2 h time block but not significantly. However, during the 2–4 h time block water consumption rebounded while alcohol consumption remained suppressed by anabasine. This indicates a more pervasive effect of anabasine on decreasing alcohol consumption.

Interestingly, anabasine at lower doses of 0.02 mg/kg and 0.2 mg/kg caused a nonsignificant trend for increasing alcohol intake. Thus, if the concentration of anabasine that a smoker derives from smoking falls within the lower doses



**Figure 4:** Anabasine effects on percent alcohol preference. Animals were pretreated with three doses of anabasine (0.02, 0.2, and 2 mg/kg, SC) or saline and their alcohol and water intake was measured at 2, 4, 6, and 24 h after the treatment. Data represent mean  $\pm$  SEM.  $N = 16$ .

used in this study, this implies that there might be an additive or synergistic interaction between anabasine and nicotine in enhancing alcohol intake. However, the pharmacological effects of higher doses of anabasine in suppressing alcohol intake might eventually be of clinical relevance.

To the best of our knowledge this is the first study on the effects of anabasine on alcohol intake. Although the mechanism of its action on alcohol intake is unclear, based on its chemical structure and its properties we can speculate that it exerts its action through nAChRs in the midbrain. Anabasine is a partial agonist at  $\alpha 4\beta 2$  nAChRs with lower affinity than nicotine, but it has a greater affinity than nicotine for the  $\alpha 7$  subtype of nAChRs, at which it is a full agonist and hypothesized to exert most of its effects in vivo [10,22]. Both  $\alpha 4\beta 2$  and  $\alpha 7$  subtypes of nAChRs have been shown to play an important role in regulating alcohol intake [11]. Anabasine may exert its effect on alcohol intake by producing partial desensitization of these receptors. It has been shown that another compound, sazetidine-A, which also desensitizes  $\alpha 4\beta 2$  nAChRs, and varenicline as well as cytisine, partial agonists at  $\alpha 4\beta 2$  containing nAChRs, reduce alcohol intake [7,18,34,37,38], suggesting that the VTA nAChRs are at least partially involved in regulation of alcohol intake [36]. Anabasine, similar to nicotine [40], has been shown to increase midbrain dopamine levels [15]. Furthermore, it has been demonstrated that anabasine substitutes for both nicotine [15] and *d*-methamphetamine [13]. Both drugs similar to alcohol stimulate midbrain dopamine release. Thus, one can speculate that anabasine, as a nicotinic partial agonist, by desensitizing nicotinic receptors located at the dopaminergic terminals in the VTA stimulates dopamine release which consequently results in reduction of alcohol

intake. In addition, anabasine's more prominent effects at  $\alpha 7$  nicotinic receptors may also differentiate it from nicotine.

The tobacco nicotinic alkaloid anabasine holds promise for development of a treatment to suppress alcohol consumption. The same anabasine dose effective in the current study (2 mg/kg) has recently been shown to significantly reduce nicotine self-administration in rats [17]. That effect could have either resulted from blockade of nicotine-induced reinforcement or substitution for nicotine effects. In our study, nicotine was not used, so there was not a nicotinic substitution possibility. The lowering of alcohol consumption by the higher dose of anabasine treatment seemed to be more likely an attenuation of alcohol's reinforcing effects or making alcohol aversive through an unknown mechanism. Anabasine and related nicotinic alkaloids should be closely studied to determine whether they may be effective new treatments for alcohol use disorders as well as tobacco addiction.

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