

Effects of Mecamylamine on Alcohol Consumption and Preference in Male C57BL/6J Mice

Justin M. Farook^a Ben Lewis^a Justin G. Gaddis^a John M. Littleton^b
Susan Barron^a

Departments of ^aPsychology and ^bPharmaceutical Sciences, College of Pharmacy, University of Kentucky, Lexington, Ky., USA

Key Words

Alcohol consumption · Preference · Mecamylamine · C57BL/6J mice

Abstract

Alcohol and nicotine (in the form of tobacco) are 2 commonly used recreational drugs and studies show a high correlation between tobacco use and alcohol consumption. In the present study, using C57BL/6J mice, we investigated the ability of mecamylamine (a nicotinic antagonist) to reduce alcohol consumption and alcohol preference with free 24-hour access using a 2-bottle choice test drinking procedure. Male C57BL/6J mice were individually housed and acclimatized to 10% alcohol. Immediately following the last day of alcohol acclimatization, the mice ($n = 5/\text{group}$) received subcutaneous injections of mecamylamine (0.5, 1 and 2 mg/kg) or saline consisting of either intermittent (3 injections given every other day) or daily (injections on all 5 days) exposures. Fluid consumption (alcohol and water) was recorded daily. The results showed that mecamylamine significantly reduced alcohol consumption and alcohol preference in both phases of intermittent and daily drug exposures, while the total fluid consumption was unchanged. These results provide further support that mecamylamine is effective in re-

ducing alcohol consumption and preference, and nicotinic-receptor-based drugs could further be explored as potential treatments for alcoholism.

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Introduction

A high correlation exists between cigarette smoking and alcohol consumption [1]. The prevalence of smoking among alcoholics is as high as 90% compared to 30% among the general population, and people who drink alcohol are 2-fold more likely to smoke than the general population [2]. Nicotine is the primary constituent found in tobacco that reinforces the pleasure of smoking. It is widely understood that the rewarding properties of nicotine are mediated through nicotinic acetylcholine receptors (nAChRs), which are primarily located in the central nervous system [3, 4]. The codependence on nicotine and alcohol is common, suggesting that there may be some shared mechanism.

B.L. and J.G.G. contributed equally to this work.

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Justin M. Farook, PhD
Department of Psychology
University of Kentucky
Lexington, KY 40506 (USA)
Tel. +1 859 797 1331, Fax +1 859 323 1979, E-Mail justinfarook@uky.edu

It is known that alcohol alters the function of neuronal nAChRs [5, 6]. For example, alcohol may increase ($\alpha 4\beta 2$) or inhibit ($\alpha 7$) nAChR receptor function [7], or blocks the discriminative stimulus properties of nicotine in rats [8]. Acute intraventricular or intra-accumbens infusion of nicotine has been shown to decrease alcohol consumption [9, 10], and this suppression of alcohol intake by nicotine involved only intermittent doses [11]. Conversely, daily administration of nicotine is known to increase alcohol consumption [12]. Administration of mecamylamine (a nicotinic antagonist) has been shown to reduce alcohol consumption [13–15]. Therefore, the objective of the present study was to evaluate if alcohol consumption could differ on intermittent versus daily administration of mecamylamine in C57BL/6J mice.

'Preference drinking' is one of the mouse models widely used in alcohol research [16]. In this model, known as the 2-bottle choice test procedure, animals are given access to 2 bottles, one with a diluted solution of alcohol and the other with plain water. Consumption is then monitored every 24 h over several days so as to make an assessment of a subject's preference for alcohol over water. The genotype of animal/strain exerts a high influence in its tendency to prefer alcohol to water. Using this 2-bottle choice test procedure, several mouse lines have been classified as 'highly alcohol preferring', or 'rarely alcohol preferring'. The C57BL/6J (B6) strain of mice is widely known to voluntarily consume significant quantities of alcohol and therefore is classified as an 'alcohol-preferring' mouse strain [17]. The B6 mouse could be of great value in investigating the pharmacological profiles of compounds thought to mediate alcohol consumption, considering the fact that a great deal of established pharmacological, behavioral and genetic resources using this inbred mouse line is already known [17]. A 2-bottle choice test procedure is currently being used in our laboratory as the model to evaluate the therapeutic potential of novel drugs in treating alcoholism [18]. The overall objective of this study was to test the effects of intermittent and daily administration of mecamylamine for their ability to attenuate alcohol consumption and alcohol preference in C57BL/6J mice.

Materials and Methods

Animals

Male C57BL/6J mice ($n = 5/\text{group}$) weighing 27–33 g (Jackson Laboratory, Bar Harbor, Me., USA) were housed 1/cage in a room maintained on a 15/9 light/dark cycle (lights on at 06:00 AM and off at 09:00 PM) and kept at a constant temperature with free ac-

cess to food and water for 7 days upon their arrival. All research was conducted in accordance with the guidelines of the National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH publications No. 80-23 revised in 1996) and approved by the University of Kentucky Institutional Animal Use and Care Committee.

Drugs

Mecamylamine (2-(methylamino)isocamphane hydrochloride N,2,3,3-tetramethylbicyclo[2.2.1]heptan-2-amine hydrochloride; $C_{11}H_{21}N \cdot HCl$) was obtained from Sigma-Aldrich, Saint Louis, Mo., USA. The drug was freshly prepared by dissolving in sterile water and injected subcutaneously at doses of 0.5, 1 and 2 mg/kg (injection volume = 10 ml/kg). These doses were chosen based on previous literature [19]. The control animals received subcutaneous saline injections in a similar injection volume.

Behavioral Procedure

Two-Bottle Choice Test Procedure: Alcohol Priming. Prior to the start of the mecamylamine administration, the mice were given 24-hour access to water and alcohol for 7 days using 2 feeding tubes (Dyets Inc., Bethlehem, Pa., USA) inserted directly into the animal cage. Over these 7 days, the concentration of alcohol was gradually increased using the following schedule: days 1–2: 3%; days 3–5: 6%; days 6–7: 10%. The position of the water and alcohol tubes was changed on alternate days in order to avoid potential position bias. Intermittent and daily administration phases were considered separate experiments and involved a separate batch of animals. Intermittent drug administration involved 3 drug injections (on days 1, 3 and 5), whereas daily administration involved drug injections on all 5 days. Every day between 14:00 and 16:00 h, the amount of alcohol and water consumed was measured for 5 days (every 24 h). Following the drug administration, the animals were put into clean cages with fresh alcohol and water. For the intermittent drug exposure group, during the days when the animals did not receive drug injections, they were put into clean cages with fresh alcohol and water immediately after measuring the amount of alcohol and water consumed. Alcohol preference was calculated by using the following equation: preference (percent) = alcohol intake \times 100/total fluid intake.

Results

Effects of Intermittent Administration of Mecamylamine

Alcohol Consumption. Mecamylamine at doses 1 and 2 mg/kg reduced alcohol consumption on days 1–2 ($p < 0.05$), at doses 0.5 ($p < 0.05$), 1 and 2 mg/kg ($p < 0.001$) reduced alcohol consumption on day 3, and at doses 0.5, 1 and 2 mg/kg reduced alcohol consumption on days 4–5 ($p < 0.001$). Two-way ANOVA for alcohol consumed: dose, $F(3, 97) = 67.927$, $p < 0.001$; day, $F(4, 97) = 6.482$, $p < 0.001$, and interaction, $F(12, 97) = 1.038$, $p = 0.424$ (fig. 1a).

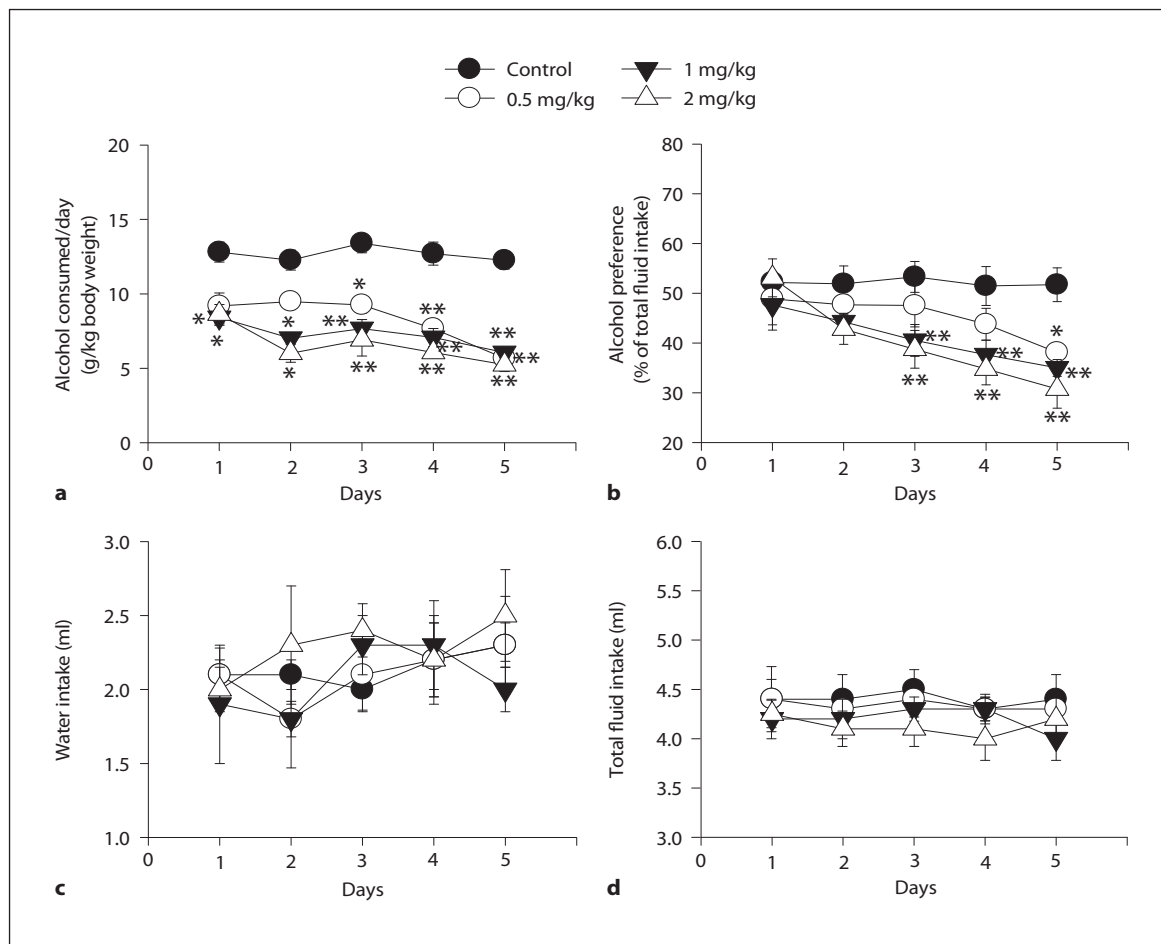


Fig. 1. Effects of subcutaneous intermittent administration of mecamylamine (doses of 0.5, 1 and 2 mg/kg) in the 2-bottle choice test procedure. **a** Alcohol consumption. **b** Alcohol preference. **c** Water intake. **d** Total fluid intake. * $p < 0.05$, or ** $p < 0.001$ by post hoc Tukey honestly significant difference against the appropriate control group across days.

Alcohol Preference. The results showed that mecamylamine at dose 0.5 mg/kg reduced alcohol consumption on day 5 ($p < 0.05$), and at doses 1 and 2 mg/kg on days 3–5 ($p < 0.001$). Two-way ANOVA for alcohol preference: dose, $F(3, 95) = 10.658$, $p < 0.001$; day, $F(4, 95) = 7.016$, $p < 0.001$, and interaction, $F(12, 95) = 1.229$, $p = 0.279$ (fig. 1b).

Water Intake. There were no statistically significant differences in water intake across treatment conditions (fig. 1c). Two-way ANOVA for water intake: dose, $F(3, 99) = 0.229$, $p = 0.570$; day, $F(4, 99) = 0.908$, $p = 0.463$, and interaction, $F(12, 99) = 0.374$, $p = 0.969$.

Total Fluid Intake. There were also no statistically significant differences in total fluid intake across days (fig. 1d). Two-way ANOVA for total fluid intake: dose,

$F(3, 97) = 1.651$, $p = 0.185$; day, $F(4, 97) = 0.278$, $p = 0.891$, and interaction, $F(12, 97) = 0.286$, $p = 0.990$.

Effects of Daily Administration of Mecamylamine

Alcohol Consumption. The results showed that mecamylamine at doses 0.5, 1 and 2 mg/kg reduced alcohol consumption on day 2 ($p < 0.05$), at doses 0.5 ($p < 0.05$), 1 and 2 mg/kg ($p < 0.001$) reduced alcohol consumption on day 3, and at doses 0.5, 1 and 2 mg/kg reduced alcohol consumption on days 4–5 ($p < 0.001$) (fig. 2a). Two-way ANOVA for alcohol consumed: dose, $F(3, 97) = 73.715$, $p < 0.001$; day, $F(4, 97) = 12.325$, $p < 0.001$, and interaction, $F(12, 97) = 3.133$, $p < 0.001$.

Alcohol Preference. The results showed that mecamylamine at doses 0.5, 1 and 3 mg/kg reduced alcohol con-

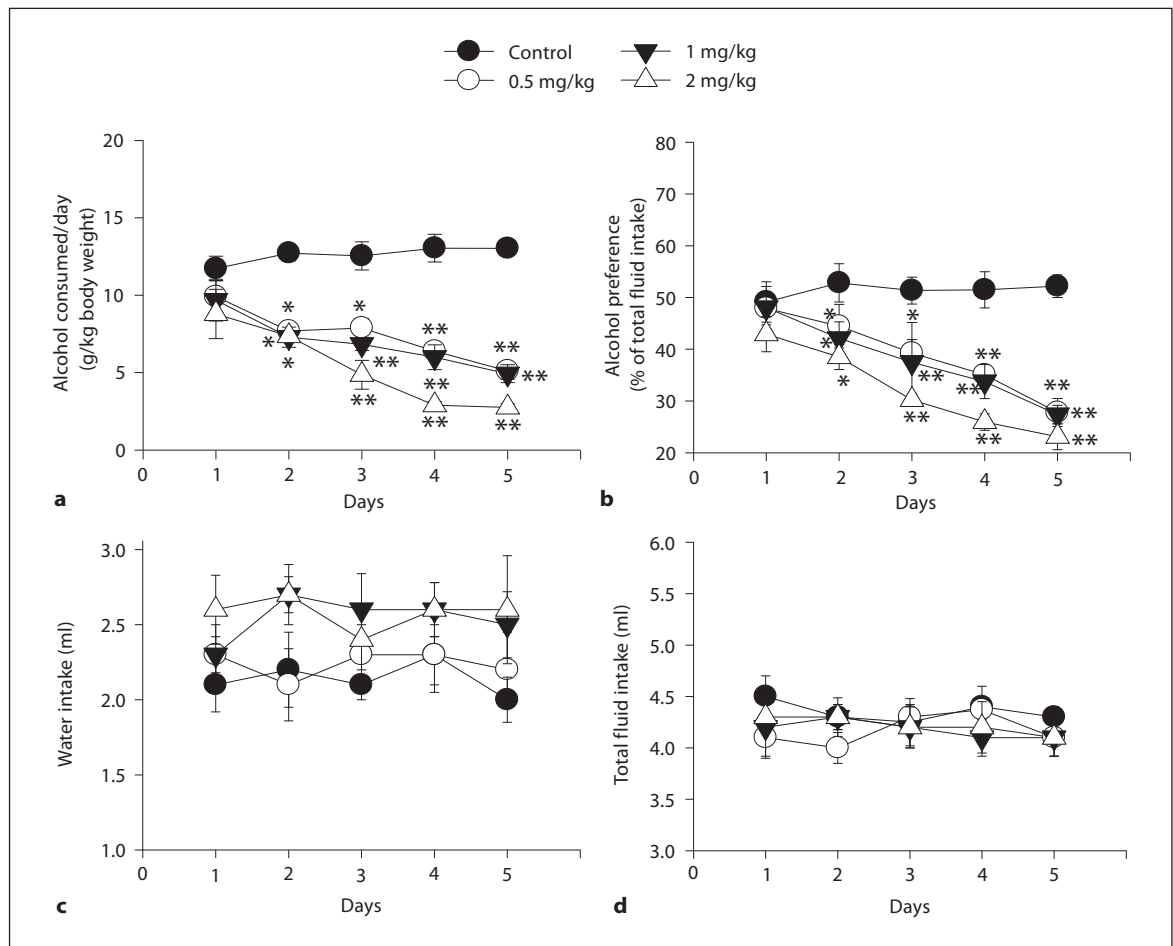


Fig. 2. Effects of subcutaneous daily administration of mecamylamine (doses of 0.5, 1 and 2 mg/kg) in the 2-bottle test choice procedure. **a** Alcohol consumption. **b** Alcohol preference. **c** Water intake. **d** Total fluid intake. * $p < 0.05$, or ** $p < 0.001$ by post hoc Tukey honestly significant difference against the appropriate control group across days.

sumption on day 2 ($p < 0.05$), and thereafter at doses 0.5, 1 and 3 mg/kg on days 3–5 ($p < 0.001$) (fig. 2b). Two-way ANOVA for alcohol preference: dose, $F(3, 95) = 25.762$, $p < 0.001$; day, $F(4, 95) = 10.709$, $p < 0.001$, and interaction, $F(12, 95) = 1.669$, $p = 0.091$.

Water Intake. There was no statistically significant difference in water intake across days (fig. 2c). Two-way ANOVA for water intake: dose, $F(3, 98) = 5.080$, $p < 0.05$; day, $F(4, 98) = 0.279$, $p = 0.891$, and interaction, $F(12, 98) = 0.305$, $p = 0.987$.

Total Fluid Intake. There was no statistically significant difference in total fluid intake across days (fig. 2d). Two-way ANOVA for total fluid intake: dose, $F(3, 97) = 0.950$, $p = 0.421$; day, $F(4, 97) = 0.274$, $p = 0.881$, and interaction, $F(12, 97) = 0.359$, $p = 0.974$.

Discussion

Alcohol and nicotine are frequently coabused, and therefore it is of interest to investigate whether alcohol consumption could be altered after the administration of a nicotinic drug. Specifically, we examined whether mecamylamine (a nicotine antagonist), administered intermittently or daily, could have an effect on alcohol consumption and alcohol preference. The results showed that mecamylamine significantly attenuated alcohol consumption and preference when administered both intermittently and daily. More importantly, a pronounced reduction in alcohol consumption and preference was noticed following daily administration of mecamylamine. Throughout these studies, the total fluid intake following

drug administration was not significantly different from baseline consumption. This eliminates any possibility that differences in alcohol preference may have been due to a sedative effect of the drug. In the present study, mecamlamine was given at doses that did not have any adverse effect on locomotor activity, a finding that has been substantiated by others [19].

The effects of mecamlamine outlined in the present study are in agreement with other studies, both in animals and in humans, which suggest that mecamlamine decreases alcohol preference and consumption in animals [13] and in human subjects [20]. In humans, studies by Blomqvist et al. [20] used 20 human subjects that were pretreated with various doses of mecamlamine (7.5–12 mg/kg) or saline followed by exposure to alcohol. The results showed that mecamlamine significantly attenuated alcohol consumption. Previous studies in rodents indicate that mecamlamine when administered systemically or directly into the ventral tegmental area blocks the elevation of alcohol-mediated dopamine release in the nucleus accumbens [21, 22]. From this inference, it is likely that in the present study, mecamlamine is reducing alcohol and preference intake via a similar mechanism. Considering the stimulant and reinforcing properties of alcohol, the findings of the present study could also suggest that mecamlamine attenuates the reinforcing properties of alcohol such as alcohol consumption and alcohol preference. It is of interest to note that less alcohol consumption was observed in mecamlamine-treated (intermittent) mice than in control mice on each of the days, including days 2 and 4 when mecamlamine was not administered. One possibility for this could be due to the sensitization of nAChRs. This hypothesis is supported by reports that suggest the $\alpha 7$ nAChRs display functional properties very different from those of the $\alpha 4\beta 2$ nAChR, such as a very fast desensitization effect, or higher Ca^{2+} permeability [23, 24].

Mecamlamine is reported to act as a noncompetitive antagonist of the NMDA receptors due to its action at the phencyclidine site [25]. For example, studies by Blomqvist et al. [21] demonstrated that mecamlamine did not have a significant effect on mesoaccumbal dopamine flow, which could suggest that mecamlamine-sensitive receptors may not influence mesoaccumbal dopamine. However, it should be noted that mecamlamine is a nonselective and noncompetitive NMDA receptor antagonist [25]. Therefore, the possibility that mecamlamine could partly have reduced alcohol consumption through nonnicotiner-gic pathways cannot be completely ruled out. The results of the present study, combined with that of a previous report [26], support the notion that nAChRs could have a multifaceted interaction at the receptor levels, particularly those associated with the psychopathology of addictive behaviors as this is evident by the ability of mecamlamine to attenuate alcohol consumption over a period of days.

Conclusion

The data presented here indicate that the intermittent and daily subcutaneous administration of mecamlamine over a range of doses attenuates the voluntary consumption of alcohol in a 2-bottle choice drinking procedure. This suggests that nAChRs may play a role in alcohol-seeking behaviors in animals having a preference for alcohol consumption.

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