The Effects of Sazetidine-A on Alcohol and Nicotine Consumption in Mice

Margaret M. Mason, Jillienne C. Touchette, Kyu Y. O'Rourke, and Anna M. Lee
Department of Pharmacology, University of Minnesota – Twin Cities
University of Minnesota

Background

- Alcohol and nicotine are two of the most commonly used drugs in the world, and are frequently co-abused. Studies have demonstrated that sazetidine-A, a pre-clinical drug that targets nicotinic acetylcholine receptors, reduces alcohol and nicotine consumption in male rat self-administration models[1,2]. The effect of sazetidine-A in mice and in female animals has not yet been tested. In this study, the effect of sazetidine-A on single-drug or co-consumption of alcohol and nicotine in male and female mice was investigated. Using an oral consumption model previously developed in Anna Lee's lab, mice consumed alcohol and/or nicotine voluntarily, which more accurately represents human consumption compared to studies in which the drugs are directly injected into the animals.
- We hypothesized that sazetidine-A would reduce consumption of both alcohol and nicotine in male and female mice in the 2-bottle or 3-bottle choice experiments. We found that in all experiments, sazetidine-A reduced overnight alcohol consumption but did not affect overnight nicotine consumption. The results of this study highlight that nicotinic acetylcholine receptors may play a different role in alcohol versus nicotine consumption, and could potentially contribute to the discovery of novel therapies for the treatment of alcohol addiction.

1. Levin, E.D., et al. (2009). Sazetidine-A, a selective α4β2 nicotinic receptor desensitizing agent and partial agonist, reduces nicotine self-administration in rats. *National Center for Biotechnology Information,* 332(3), pp. 993-999.
2. Rezvani, A.H., et al. (2010). Effects of sazetidine-A, a selective α4β2 nicotinic acetylcholine receptor desensitizing agent on alcohol and nicotine self-administration in selectively bred alcohol-preferring (P) rats. *National Center for Biotechnology Information,* 211(2), pp. 161-174.

Methods

- For all consumption experiments, mice were individually housed and weighed once per week throughout the study. Mice were given voluntary access to two bottles containing either water or drug. Bottles were weighed every 2 days and the bottle positions were switched at this time to account for side preferences. Three intraperitoneal saline injections were administered during the experiment to habituate the mice to injections. The controls for this experiment were empty cages without mice that contained water and drug bottles, and the drips from these bottles were measured and subtracted from the weight of all bottles for each session.

2-Bottle Choice Experiments:

- For alcohol consumption, naïve male and female mice were presented with increasing concentrations of alcohol: 3, 6, 10, 14, and 20% v/v; presented for 4 days each. After 4 weeks of drug consumption, mice received an intraperitoneal injection of sazetidine-A (1mg/kg) or saline one hour prior to the dark cycle, and bottles were measured the next morning. The following evening, mice received a second injection of sazetidine-A or saline and bottles were again measured the next morning.
- For nicotine consumption, another group of naïve male and female mice were presented with increasing concentrations of nicotine: 5, 10, 15, and 30μg/mL, presented for 2 days each. After 4 weeks of drug consumption, mice received an intraperitoneal injection of saline or sazetidine-A (1mg/kg) one hour prior to the dark cycle, and bottles were measured the next morning.

3-Bottle Choice Experiment:

- Drug naïve male and female mice were presented with intermittent access to increasing concentrations of alcohol, nicotine, and water on Mondays, Wednesdays, and Fridays. Mice had access to only water on all other days. The concentrations of the drug bottles were escalated during week 1 (see 2-bottle choice methods for concentrations). After 4 weeks of consumption, mice received an intraperitoneal injection of sazetidine-A (1mg/kg) or saline one hour prior to the dark cycle, and bottles were measured the next morning. The following evening, mice received a second injection of sazetidine-A or saline and bottles were again measured the next morning.

Data Analysis:

- After consumption results were recorded, drug consumption was compared between animals injected with saline versus animals injected with sazetidine-A. The sexes of mice were analyzed individually due to the variation in consumption between males and females. Statistical analysis was performed using two-way repeated measure ANOVA or Student's *t*-test using Prism 6.0. Results were considered significant if $P<0.05$.

Summary and Conclusions

- In single-drug and co-consumption experiments, sazetidine-A reduces alcohol but not nicotine consumption in both male and female mice.
- Our data suggest that nicotinic acetylcholine receptors may mediate alcohol and nicotine consumption by separate mechanisms.

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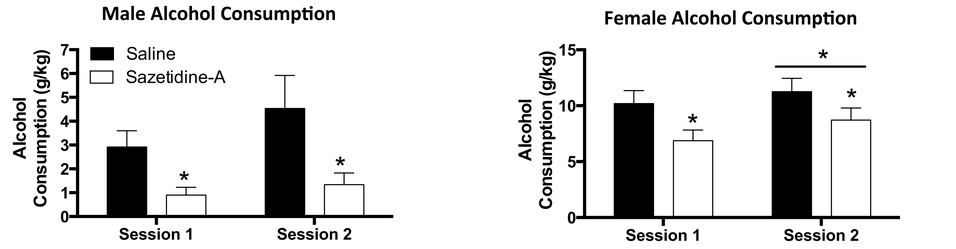
Sazetidine-A reduces alcohol consumption in a continuous access 2-bottle choice test

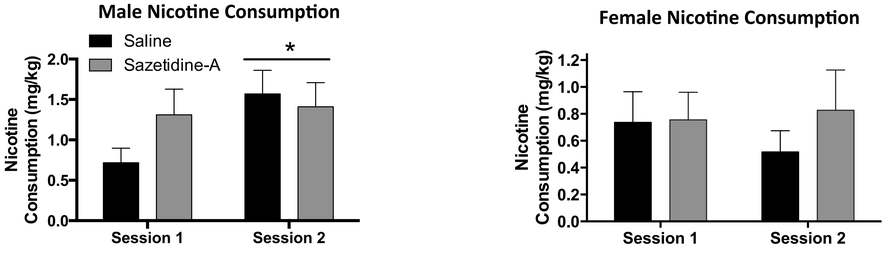


$F_{(treatment)}(1,13)=28.04$, $P=0.02$

$F_{(treatment)}(1,47)=6.91$, $P=0.048$; $F_{(session)}(1,47)=1.71$, $P=0.015$

*$P<0.05$, for an overall effect of treatment or session in a two-way RM ANOVA. Males n=7-8, females n=23-26 C57BL/6 mice.

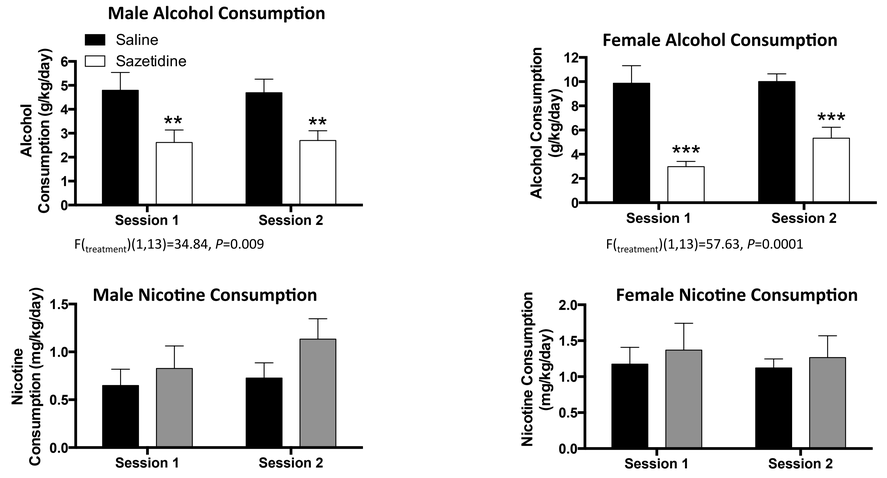
Sazetidine-A does not reduce nicotine consumption in a continuous access 2-bottle choice test



$F_{(session)}(1,18)=7.12$, $P=0.025$

*$P<0.05$, for an overall effect of session in a two-way RM ANOVA. Males n=10 C57BL/6 mice, Females n=7 C57BL/6x129 mice.

Sazetidine-A reduces alcohol but not nicotine consumption in an intermittent access 3-bottle choice test



$F_{(treatment)}(1,13)=34.84$, $P=0.009$

$F_{(treatment)}(1,13)=57.63$, $P=0.0001$

**$P<0.01$, ***$P<0.001$ for an overall effect of treatment in a two-way RM ANOVA. n=7 saline, n=8 Sazetidine-treated C57BL/6 mice