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The nicotinic receptor drug sazetidine-A reduces alcohol consumption in mice without affecting concurrent nicotine consumption

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ABSTRACT

Alcohol and nicotine addiction are frequently co-morbid. The nicotinic acetylcholine receptors (nAChRs) are critical for both alcohol and nicotine addiction mechanisms, since nAChR drugs that reduce nicotine consumption have been shown to also reduce alcohol consumption. Sazetidine-A, a pre-clinical nAChR drug with agonist and desensitizing effects at α4β2 and α7 nAChRs, has been reported to reduce alcohol consumption and nicotine self-administration in rats when administered at high doses. However, this effect has not been replicated in mice. In this study, we examined the effect of sazetidine-A on alcohol and nicotine consumption in male and female mice utilizing voluntary oral consumption procedures previously developed in our lab. We found that sazetidine-A (1 mg/kg, *i.p*) reduced overnight alcohol consumption, but did not affect nicotine consumption when presented either alone or concurrently with alcohol. Sazetidine-A did not reduce water or saccharin consumption at any dose tested. In a chronic co-consumption experiment in which either alcohol or nicotine was re-introduced after one week of forced abstinence, sazetidine-A attenuated post-abstinence consumption of alcohol but not nicotine. Sazetidine-A also significantly reduced alcohol consumption in an acute, binge drinking-in-the-dark procedure. Finally, we tested the effect of sazetidine-A on alcohol withdrawal, and found that sazetidine-A significantly reduced handling-induced convulsions during alcohol withdrawal. Collectively, these data suggest a novel role for the nAChR targets of sazetidine-A in specifically mediating alcohol consumption, separate from the involvement of nAChRs in mediating nicotine consumption. Delineation of this pathway may provide insight into novel therapies for the treatment of alcohol use disorders.

1. Introduction

Nicotine and alcohol are two of the most widely used addictive drugs, and their use is frequently co-morbid. Alcohol abuse is much more common among smokers compared with non-smokers, and up to 80–90% of people with alcohol dependence also smoke cigarettes (Anthony and Echeagaray-Wagner, 2000; Batel et al., 1995; DiFranza and Guerrera, 1990). Additionally, numerous epidemiological reports have demonstrated that the use of either alcohol or nicotine increases the use of the other drug (Barrett et al., 2006; Chen et al., 2002; Harrison et al., 2008; Weitzman and Chen, 2005). Nicotine and alcohol both mediate their reinforcing properties by modulating the release of dopamine (DA) from ventral tegmental (VTA) neurons that project to the nucleus accumbens (NAc) (Balfour et al., 1998; Gessa et al., 1985; Mifsud et al., 1989; Weiss et al., 1993). Nicotine and alcohol addiction are heritable and share common genetic factors and neuromolecular mechanisms, including the involvement of the nicotinic acetylcholine receptors (nAChRs) (Hendrickson et al., 2013; Li and Burmeister, 2009; Li et al., 2003).

The nAChRs are ligand-gated cation channels that are activated by the endogenous ligand acetylcholine (for review, see (Albuquerque et al., 2009)). Eleven nAChR subunits are expressed in the brain (α2-7, α9-10, β2-4) that combine to form pentameric channels with distinct pharmacological properties and physiological functions (Albuquerque et al., 2009; Wonnacott et al., 2006).

Abbreviations: nAChR, nicotinic acetylcholine receptor; DA, dopamine; VTA, ventral tegmental area; NAc, nucleus accumbens; DHβE, dihydro-beta-erythroidine; 5-HT_3, 5-hydroxytryptamine3; KO, gene knock-out; HIC, handling-induced convulsions.

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The nAChRs are primarily located on neuronal cell bodies and presynaptic terminals, and thus are positioned to directly modulate cell excitability in the mesolimbic reward circuitry. Activation of nAChRs excites VTA dopaminergic neurons to initiate DA release in the NAc. The nAChRs are well-known to play a critical role in nicotine reinforcement (for reviews, see (Changeux, 2010; de Kloet et al., 2015)). Genetic and pharmacological evidence also strongly implicate a role for the nAChRs in alcohol addiction (for reviews, see (Chatterjee and Bartlett, 2010; Davis and de Fiebre, 2006; Hendrickson et al., 2013)), but the identity of the specific nAChR subtypes that are critical for alcohol addiction remain unclear. Unlike nicotine, alcohol does not directly activate nAChR channels, but alters ligand-induced nAChR currents, depending on the subunit composition of the receptor (Covernton and Connolly, 1997; Nagata et al., 1996; Yu et al., 1996). Additionally, voluntary alcohol consumption increases acetylcholine levels in the VTA in rats, (Larsson et al., 2005), and cholinergic signaling through nAChRs contributes to DA release in the NAc and to alcohol reward and reinforcement in rats and mice (Blomqvist et al., 1993, 1996; Kuzmin et al., 2009; Soderpalm et al., 2000). Therefore, cholinergic signaling through nAChRs is a critical component of alcohol addiction.

Most nAChR-acting drugs that reduce nicotine consumption are also capable of reducing alcohol consumption. The non-specific nAChR antagonist mecamylamine consistently reduces nicotine consumption (Corrigall and Coen, 1989; DeNoble and Mele, 2006; Glick et al., 1996; Rose et al., 1994), decreases alcohol consumption and preference in rodents (Blomqvist et al., 1996, 2002; Farook et al., 2009; Ford et al., 2009; Hendrickson et al., 2009) and blocks alcohol-induced DA release in the NAc (Blomqvist et al., 1993, 1997; Ericson et al., 1998). Cytisine, an α4β2 partial agonist, has also been shown to reduce both alcohol and nicotine consumption in mice (Hendrickson et al., 2009). More recently, varenicline, an α4β2 partial agonist developed for smoking cessation, was found to reduce both nicotine and alcohol consumption in mice (Hendrickson et al., 2010), rats (Chatterjee et al., 2011; Steensland et al., 2007), and humans (Litten et al.; McKee et al., 2009; Mitchell et al., 2012). The mechanism by which mecamylamine and varenicline reduce alcohol consumption and the specific nAChRs required for this effect remains unclear. In contrast, dihydro-beta-erythroidine (DHβE), a competitive antagonist of β2- and β4-containing nAChRs, attenuates the behavioral effects of nicotine *in vivo* (Damaj et al., 1995; Walters et al., 2006; Watkins et al., 1999), but does not reduce alcohol consumption (Kuzmin et al., 2009; Le et al., 2000). These pharmacological data coincide with studies of mice with genetic deletions for the α4 or β2 nAChR subunit, which show that α4 and β2 knock-out (KO) mice do not self-administer nicotine (Picciotto et al., 1998; Pons et al., 2008), and that α4, but not β2, knock-out mice consume less alcohol compared to wild-type mice (Dawson et al., 2013; Hendrickson et al., 2011). Therefore, different nAChRs subtypes appear to have differential roles in mediating nicotine-specific and alcohol-specific mechanisms.

Sazetidine-A is a pre-clinical nAChR drug that has recently been shown to reduce alcohol consumption and *i.v.* nicotine self-administration in rats (Johnson et al., 2012; Levin et al., 2010; Rezvani et al., 2010). Similar to other nAChR drugs, the mechanism by which sazetidine-A reduces alcohol consumption is unclear. Sazetidine-A was originally described as a "silent desensitizer" of α4β2 nAChRs (Xiao et al., 2006), but has since been discovered to have differential specificity depending on the subunit composition of the α4β2 pentamer. Sazetidine-A fully activates the $\alpha4_2\beta2_3$ stoichiometry (Xiao et al., 2006; Zwart et al., 2008) and has negligible activity at $\alpha4_3\beta2_2$ nAChRs (Campling et al., 2013; Eaton et al., 2014; Zwart et al., 2008), due to the selectivity of sazetidine-A at the α4/β2 but not the α4/α4 interface (Eaton et al., 2014; Wang et al., 2015; Zwart et al., 2008). In addition, sazetidine-A has full agonist and desensitizing activity at α7 nAChRs (Brown and Wonnacott, 2015), full agonist activity at α3β4* nAChRs, and partial agonist activity at α4β4* and α6β2* receptors (* denotes the possible presence of additional subunits in the receptor pentamer) (Campling et al., 2013; Kuryatov and Lindstrom, 2011). Like nicotine, sazetidine-A causes long-term desensitization of nAChRs that can potentially last for hours (Campling et al., 2013; Eaton et al., 2014; Paradiso and Steinbach, 2003). Moreover, sazetidine-A accumulates in the brain and can occupy β2-containing nAChRs with a dissociation half-life of 8–24 h (Caldarone et al., 2011).

In rat studies showing that sazetidine-A reduces alcohol and nicotine consumption, the doses used also caused reduced consumption of food and reduced intake of saccharin solution (Rezvani et al., 2010), suggesting potential off-target effects of sazetidine-A. Additionally, the effect of sazetidine-A has never been tested in mice, nor on voluntary, oral co-consumption of alcohol and nicotine presented concurrently. In this study, we tested the effect of sazetidine-A on alcohol and nicotine consumption in male and female mice using models of voluntary, oral consumption that we developed and have described previously (O'Rourke et al., 2016). Here, we show for the first time that sazetidine-A, at a dose that does not affect water or saccharin consumption, reduces alcohol consumption in mice without affecting nicotine consumption. We found that sazetidine-A also reduced handling-induced convulsions during alcohol withdrawal, revealing a potential mechanism by which sazetidine-A reduces alcohol consumption.

2. Materials and methods

2.1. Animals and drugs

Adult male and female mice a minimum of 8 weeks old were used in all experiments. C57BL/6 mice were purchased from Jackson Laboratory (Sacramento, CA) and acclimated to the facility for a minimum of six days before behavioral experiments. All mice were group housed under a standard 12 h light/dark cycle until the start of experiments, after which they were individually housed. All animal procedures were performed in accordance with the Institutional Animal Care and Use Committee at the University of Minnesota, and conformed to NIH guidelines.

Alcohol (Decon Labs, King of Prussia, PA), and nicotine tartrate salt (Acros Organics, Thermo Fisher Scientific, Chicago, IL) were mixed with tap water to the concentrations reported for each experiment. The concentrations of nicotine are reported as free base, and nicotine solutions were not filtered or pH adjusted. Neither the alcohol nor nicotine solutions contained sweetener. Sazetidine-A dihydrochloride was purchased from Tocris Bioscience (Bio-Techne, Minneapolis, MN), and made fresh for each experiment by dissolving in 0.9% saline and was not filtered or pH adjusted.

2.2. Experiment 1A and 1B: the effect of sazetidine-A on water and saccharin consumption

Experiment 1A: Drug naïve male C57BL/6 mice were singly housed and underwent sequential experiments to first assess the effect of sazetidine-A on consumption of water only, followed by saccharin consumption in a continuous 24-h access two-bottle choice procedure. Food and water were freely available at all times throughout the procedures. For the water consumption portion, one bottle of water was available at all times. Three intraperotineal (*i.p.*) saline injections were administered on