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Knockout of alpha 5 nicotinic acetylcholine receptors subunit alters ethanol-mediated behavioral effects and reward in mice

Anton Dawson [a], Jennifer T. Wolstenholme [a], Monzurul A. Roni [b], Vera C. Campbell [b], Asti Jackson [a], Cassandra Slater [a], Deniz Bagdas [a], Erika E. Perez [c], Jill C. Bettinger [a], Mariella De Biasi [c], Michael F. Miles [a], M. Imad Damaj [a, *]

[a] Department of Pharmacology and Toxicology, Virginia Commonwealth University School of Medicine, Richmond, VA, 23298-0613, USA
[b] Department of Pharmaceutical Sciences, Hampton University School of Pharmacy, Hampton, VA, 23668, USA
[c] Department of Psychiatry, Perelman School of Medicine at the University of Pennsylvania, Philadelphia, PA, 19104, USA

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ABSTRACT

Evidence suggests that there is an association between polymorphisms in the α5 nicotinic acetylcholine receptor (nAChR) subunit and risk of developing alcohol dependence in humans. The α5 nAChR subunit has also recently been shown to modulate some of the acute response to ethanol in mice. The aim of the current study was to further characterize the role of α5-containing (α5*) nAChRs in acute ethanol responsive behaviors, ethanol consumption and ethanol preference in mice. We conducted a battery of tests in male α5 knockout (KO) mice for a range of ethanol-induced behaviors including hypothermia, hypnosis, and anxiolysis. We also investigated the effects of α5* nAChR on ethanol reward using the Conditioned Place Preference (CPP) assay. Further, we tested the effects of gene deletion on drinking behaviors using the voluntary ethanol consumption in a two-bottle choice assay and Drinking in the Dark (DID, with or without stress) paradigm. We found that deletion of the α5 nAChR subunit enhanced ethanol-induced hypothermia, hypnosis, and an anxiolytic-like response in comparison to wild-type controls. The α5 KO mice showed reduced CPP for ethanol, suggesting that the rewarding properties of ethanol are decreased in mutant mice. Interestingly, *Chrna5* gene deletion had no effect on basal ethanol drinking behavior, or ethanol metabolism, but did decrease ethanol intake in the DID paradigm following restraint stress. Taken together, we provide new evidence that α5 nAChRs are involved in some but not all of the behavioral effects of ethanol. Our results highlight the importance of nAChRs as a possible target for the treatment of alcohol dependence.

1. Introduction

Alcohol and nicotine are two of the most commonly abused drugs, contributing to over 500,000 deaths annually in the United States, with associated medical costs in excess of $500 billion per year (Centers for Disease Control and Prevention, 2014, 2015). Multiple studies have observed that up to 90% of individuals diagnosed with alcohol use disorder are cigarette smokers, with between 20 and 40% of them being heavy smokers, defined by 40 + cigarettes per day (Istvan and Matarazzo, 1984; DiFranza and Guerrera, 1990; Batel et al., 1995; Falk et al., 2006). Interestingly, increased alcohol abstinence rates have been reported in alcoholics who attempt to quit smoking and smoking cessation was shown not to disrupt alcohol abstinence (Gulliver et al., 2007; for review, see Hughes and Kalman, 2006). Evidence from both human and animal studies supports the notion that there are common genetic factors underlying these disorders (Schlaepfer et al., 2008a, 2008b; Cross et al., 2017). Here, we investigate the role of the alpha 5 subunit of nicotinic acetylcholine receptor, *Chrna5*, in mouse models of ethanol addiction-related behaviors (for review, see Bühler et al., 2015).

Investigations of the *CHRNA5-CHRNA3-CHRNB4* gene cluster in humans have suggested a role for α5* nicotinic subunits in nicotine and alcohol dependence (Joslyn et al., 2008; Schlaepfer et al., 2008a, 2008b), as well as in the level of response to alcohol, which may impact liability to develop alcohol dependence. These

* Corresponding author. Department of Pharmacology and Toxicology, Virginia Commonwealth University, Box 980613, Richmond, VA, 23298-0613, USA.
E-mail address: m.damaj@vcuhealth.org (M.I. Damaj).

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studies have implicated single nucleotide polymorphisms (SNPs) in the CHRNA5 gene, encoding the α5 nAChR subunit, to be associated with an increased risk to develop alcohol dependence (Joslyn et al., 2008; Schlaepfer et al., 2008a, 2008b; Choquet et al., 2013). Furthermore, human genome-wide association studies have associated a promoter single nucleotide polymorphism (SNP) in *CHRNA5,* rs588765, with alcohol dependence in European Americans. Individuals homozygous for this SNP had significantly higher mRNA levels of *CHRNA5* in the frontal cortex than heterozygotes or those without the SNP (Wang et al., 2009). Mouse genetic studies have reported that there is an allelic difference in *Chrna5* between C57BL/6J and DBA/2J (alcohol-avoiding mice) that can be associated with alcohol preference in the strains (Symons et al., 2010). Furthermore, these researchers reported that whole brain *Chrna5* mRNA levels were significantly increased in C57BL/6J mice compared to DBA/2J mice following ethanol treatment (Symons et al., 2010). Finally, using α5 knockout (KO) mice, Santos et al. (2012) study suggest that the α5 nAChR subunit is important for the sedative effects of ethanol but does not play a role in ethanol oral intake in mice. Overall, these human and mouse studies have suggested an important role for the α5 nicotinic subunit in alcohol responses and alcohol dependence.

Neuronal nicotinic acetylcholine receptors (nAChRs) are ligand-gated ion channels that form pentamers containing α (α2-α10) and/or β (β2-β4) subunits. Alpha 5 is an accessory subunit that forms pentamers with either α4β2, α3β4, or α3β2 subunits, and together, these constitute 10–37% of all nAChRs (Kuryatov et al., 2008; Baddick and Marks, 2011). Although high levels of expression are found only in few brain areas (Salas et al., 2003a), Chrna5 mRNA has been found in the majority of brain regions (Brown et al., 2007), suggesting that a5* nAChRs could have a substantial impact on brain function. For example, α5* nAChRs play a key role in nicotine intake, reward, and withdrawal in rodents (Salas et al., 2003a; Jackson et al., 2010; Jackson et al., 2008; Fowler et al., 2011: Morel et al., 2014). For example, α5 KO mice showed an enhancement of nicotine reward and intake (Jackson et al., 2010; Fowler et al., 2011) and a reduction in some nicotine wihdrawal signs (Salas et al., 2003a; Jackson et al., 2008). Expression studies in *Xenopus* oocytes also found that ethanol potentiated currents produced by acetylcholine in certain nAChR subunit combinations (α4β2*, α4β4*, α2β4*, and α2β2*), had no effect on receptors containing α3β4 or α3β2 subunits, and inhibited the function of α7 receptors, demonstrating that the effects of ethanol depend on the nAChR subtype considered (Cardoso et al., 1999; Borghese et al., 2003). A variety of ethanol's behavioral effects can be modulated by altered nAChR function (Kuzmin et al., 2008; Kamens et al., 2010, 2012; Santos et al., 2012; Sajja and Rahman, 2012). Importantly, Santos and colleagues (Santos et al., 2012), observed that mice lacking *Chrna*5 demonstrated prolonged sleep time following administration of a sedative dose of ethanol, showed greater impairment of locomotion by ethanol evidenced by a decreased latency to fall off of a rotarod, but consumed similar amounts of ethanol compared to wild-type mice in the Drinking in the Dark (DID) forced drinking model (Santos et al., 2012). These results indicate that more work is needed to fully understand the role α5* nAChRs plays in mediating phenotypes associated with alcohol dependence.

In this study, we seek to further characterize the role of the α5 nAChR subunit on ethanol responsive behaviors and rewarding effects in mice. We hypothesized that deletion of the *Chrna5* gene would result in altered responses to ethanol in a battery of behavioral tests with emphasis on phenotypes associated with risk for alcoholism and across a range of alcohol doses. Using α5 knockout (KO) and wild-type (WT) mice, we explored the role of α5* nAChRs in three acute ethanol-responsive behaviors: hypothermia, loss of righting reflex, and anxiolysis. We also examined ethanol consumption and reward in multiple mouse paradigms of oral drinking and conditioned place preference (CPP).

2. Materials and methods

2.1. Animals

The α5 KO breeding pairs were procured from The Jackson Laboratory (Bar Harbor, ME) and bred in the animal facility at Virginia Commonwealth University (Richmond, VA). They were originally reported by Salas et al. (2003a). All mice used in each experiment were backcrossed for at least 12 generations on C57BL/6J background. Heterozygote KO/+ mice were crossed to generate homozygous mutant and WT control littermates. Male mice were group-housed in a temperature and humidity controlled animal care facility approved by Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC), and had free access to food and water under a 12-h light/dark cycle (lights on at 6:00 am) schedule. All experiments were performed during the light cycle. Mice were 8–10 weeks old at the start of the experiments. The study was approved by the Institutional Animal Care and Use Committee of Virginia Commonwealth University. All studies were carried out in accordance with the United States National Institutes of Health Guide for the Care and Use of Laboratory Animals. Behavioral and pharmacological tests were performed on different cohorts of mice.

2.2. Drugs

200-proof ethanol (Pharmco-AAPER, USA) was dissolved in 0.9% saline to yield a 20% (v/v) solution and injected intraperitoneally (i.p) for acute experiments. For drinking studies, ethanol (3–30%) was administered orally (p.o.) in the drinking water. Ketamine HCl (100 mg/mL solution, KetaVed™), obtained from Vedco Inc (Saint Joseph, MO), was diluted to 10 mg/mL in saline. Sodium pentobarbital, USP (50 mg/mL), purchased from Virginia Commonwealth University Hospital Pharmacy, was diluted to 3 mg/mL in saline. Ketamine HCl and sodium pentobarbital were each administered i.p. at a volume of 0.1 mL per 10 g of mouse mass for doses of 100 mg/kg and 30 mg/kg, respectively. The doses of alcohol (2–4 g/kg) used in acute behavioral studies and conditioned place preference test were based on previously published studies (Alkana et al., 1992; Browman et al., 2000; Tanchuck-Nipper et al., 2015; Putman et al., 2016; Slater et al., 2016; Guildford et al., 2016).

2.3. Body temperature measurement

Hypothermia was measured by rectally inserting a standard thermometer probe ~24 mm (Fischer Scientific, Pittsburgh, PA). Baseline temperatures were recorded and five minutes later, mice were administered 2.5 g/kg ethanol or saline (i.p.). Body temperature was recorded 15, 30, 60, and 120 min following ethanol injection. Data were expressed as the mean temperature change (°C) ± SEM from baseline after ethanol treatment. The room temperature of the laboratory varied from 21-24 °C.

2.4. Loss of righting reflex (LORR)

The LORR assay was used to assess the sedative-hypnotic effects of ethanol, pentobarbital, and ketamine. The α5 KO and WT mice were administered i.p. injections of ethanol (3.8 g/kg), pentobarbital (30 mg/kg) or ketamine (100 mg/kg), then placed into a supine position in a V-shaped trough. A mouse was confirmed to have achieved LORR only after it remained on its back for at least 30 sec. The time from ethanol, pentobarbital or ketamine injection until