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GREATER ETHANOL INHIBITION OF PRESYNAPTIC DOPAMINE RELEASE IN C57BL/6J THAN DBA/2J MICE: ROLE OF NICOTINIC ACETYLCHOLINE RECEPTORS

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Abstract—The mesolimbic dopamine system, originating in the ventral tegmental area (VTA) and projecting to the nucleus accumbens (NAc), has been heavily implicated in the reinforcing effects of ethanol. Recent slice voltammetry studies have shown that ethanol inhibits dopamine release selectively during high-frequency activity that elicits phasic dopamine release shown to be important for learning and reinforcement. Presently, we examined ethanol inhibition of electrically evoked NAc dopamine in two mouse strains with divergent dopamine responses to ethanol, C57BL/6 (C57) and DBA/2J (DBA) mice. Previous electrophysiology and microdialysis studies have demonstrated greater ethanol-induced VTA dopaminergic firing and NAc dopamine elevations in DBA compared to C57 mice. Additionally, DBA mice have greater ethanol responses in dopamine-related behaviors, including hyperlocomotion and conditioned place preference. Currently, we demonstrate greater sensitivity of ethanol inhibition of NAc dopamine signaling in C57 compared to DBA mice. The reduced sensitivity to ethanol inhibition in DBA mice may contribute to the overall greater ethanol-induced dopamine signaling and related behaviors observed in this strain. NAc cholinergic activity is known to potently modulate terminal dopamine release. Additionally, ethanol is known to interact with multiple aspects of nicotinic acetylcholine receptor activity. Therefore, we examined ethanol-mediated inhibition of dopamine release at two ethanol concentrations (80 and 160 mM) during bath application of the non-selective nicotinic receptor antagonist mecamylamine, as well as compounds selective for the β2-(dihydro-β-erythroidine hydrobromide; DhβE) and α6-(α-conotoxin MII [H9A; L15A]) subunit-containing receptors. Mecamylamine and DhβE decreased dopamine release and reduced ethanol's inhibitory effects on dopamine in both DBA and C57 mice. Further, α-conotoxin also reduced the dopamine release and the dopamine-inhibiting effects of ethanol at the 80 mM, but not 160 mM, concentration. These data suggest that ethanol is acting in part through nicotinic acetylcholine receptors, or downstream effectors, to reduce dopamine release during high-frequency activity.

Key words: ethanol vulnerability, phasic dopamine, voltammetry, C57BL/6 DBA/2 mice, nucleus accumbens, mecamylamine.

INTRODUCTION

The mesolimbic dopamine system, which sends projections from the ventral tegmental area (VTA) to limbic structures such as the nucleus accumbens (NAc), has been implicated in the rewarding and reinforcing properties of ethanol and other drugs of abuse (for review see Sulzer, 2011). Electrophysiological and electrochemical studies have shown that acute ethanol increases VTA dopamine cell firing rates and dopamine release in the NAc (Mereu and Gessa, 1984; Imperato and Di Chiara, 1986; Brodie and Appel, 1998). Additionally, behavioral studies have shown that pharmacological or genetic manipulations which diminish dopamine activity also inhibit ethanol consumption, ethanol preference (Ikemoto et al., 1997; El-Ghundi et al., 1998; Phillips et al., 1998), conditioned place preference (CPP) for ethanol (Cunningham et al., 2000; Risinger et al., 2001; Young et al., 2013), and the acquisition of ethanol self-administration (Risinger et al., 2000).

Although it is clear that dopamine activity in the NAc is involved in ethanol reinforcement, its exact contribution has been difficult to resolve due to the opposing effects of low- vs. high-dose ethanol on dopamine neurotransmission. For example, microdialysis studies have found that intraperitoneal (I.P.) administration of low to moderate doses (1–2.5 g/kg) of ethanol results in increases in NAc dopamine levels (Imperato and Di Chiara, 1986; Yoshimoto et al., 1992; Blomqvist et al., 1993; Weiss et al., 1993; Ericson et al., 1998). These ethanol-induced increases in dopamine levels reach their peak at doses around 1 g/kg. However, at higher doses (2–5 g/kg I.P.), dopamine responses are reduced or reversed to decreases (Imperato and Di Chiara, 1986;

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Abbreviations: α-Ctx, α-conotoxin; aCSF, artificial cerebral spinal fluid; ANOVA, analysis of variance; AP-5, (2R)-amino-5-phosphonovaleric acid; C57, C57BL/6J; CPP, conditioned place preference; DBA, DBA/2J; DhβE, dihydro-β-erythroidine hydrobromide; I.P., intraperitoneal; NAc, nucleus accumbens; nAChRs, nicotinic acetylcholine receptors; VTA, ventral tegmental area.

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Blanchard et al., 1993). Voltammetry studies have also illustrated the dose-dependent, biphasic effects of ethanol. For instance, excitatory effects of ethanol have been identified using voltammetry in freely moving rats, where non-contingent ethanol administration (0.125–2.0 g/kg I.P.) increased the frequency of naturally occurring spontaneous dopamine release events (Cheer et al., 2007; Robinson et al., 2009). Furthermore, in anesthetized rats and mice, very low doses of ethanol (0.1 g/kg) increase the amplitude of electrically evoked NAc dopamine release (Yavich and Tiihonen, 2000; Pelkonen et al., 2010). However, ethanol has been shown to inhibit electrically evoked dopamine at low to high doses (0.5–5 g/kg) *in vivo* (Yavich and Tiihonen, 2000; Budygin et al., 2001a; Jones et al., 2006; Pelkonen et al., 2010) and at supraphysiological concentrations in brain slices from mice and rats (100–200 mM; Budygin et al., 2001b; Mathews et al., 2006). Together, these studies suggest that ethanol can have both excitatory and inhibitory effects on dopaminergic activity and NAc dopamine levels, depending on the dose/concentration of ethanol used in the study.

Not only are the biphasic effects of ethanol on dopamine release dose-dependent, but we have recently shown that the inhibitory effect of ethanol on dopamine release is dependent upon the frequency of stimulation (Yorgason et al., 2014). High- and low-frequency stimulations are often used to model two distinct modes of firing that occur in VTA dopamine neurons and which have differential effects on dopamine release at terminals. Tonic, low-frequency firing of VTA dopamine neurons occurs in a pacemaker fashion at 1–10 Hz (Grace and Bunney, 1984; Overton and Clark, 1997; Hyland et al., 2002; Panin et al., 2012). In the presence of salient stimuli, such as rewards or reward-predicting cues, dopamine neurons shift to a phasic firing mode with bursts of action potentials occurring at 14–22 Hz (Grace and Bunney, 1984; Overton and Clark, 1997; Hyland et al., 2002; Panin et al., 2012). We have recently shown that that dopamine release is particularly sensitive to the inhibitory effects of ethanol under high-frequency stimulation parameters in the NAc (Yorgason et al., 2014).

C57BL/6J (C57) and DBA/2J (DBA) mice are two mouse strains that have been identified for their divergent dopamine and related behavioral responses to ethanol. Although C57 and DBA mice have similar baseline VTA dopamine firing rates (Brodie and Appel, 2000; McDaid et al., 2008) and NAc dopamine levels (Kapasova and Szumlinski, 2008), they show very different sensitivities to ethanol's behavioral and neurochemical effects. While DBA mice do not consume ethanol as readily as C57s, possibly due to taste aversion in DBAs (Grahame and Cunningham, 1997; Blizard, 2007; McCool and Chappell, 2012), they exhibit greater ethanol sensitivity in dopamine-related behaviors such as CPP, hyperlocomotion, and locomotor sensitization (Cunningham and Noble, 1992; Phillips et al., 1994; Gremel et al., 2006; Melon and Boehm, 2011; Rose et al., 2013). Additionally, DBA mice are more sensitive to ethanol's excitatory effects on VTA dopamine firing activity (Brodie and Appel, 2000) and elevations in NAc dopamine levels (Kapasova and Szumlinski, 2008; but see Zapata et al., 2006). These previous electrophysiological and neurochemical studies suggest that DBA mice are more susceptible to the dopamine-increasing effects of ethanol. Since phasic dopamine release is important for ethanol reinforcement learning, and DBA mice are more susceptible to the reinforcing effects of ethanol, we hypothesized that DBA mice may be less sensitive to ethanol's inhibitory effects on dopamine release in response to high-frequency stimulations. Therefore, we examined ethanol inhibition of dopamine release in these strains across a range of stimulation frequencies.

In addition to ethanol's effects on dopamine in C57 and DBA mice, we were also interested in potential mechanisms that explain the differences in these strains. Nicotinic acetylcholine receptors (nAChRs) located on dopamine nerve terminals are known to be powerful modulators of dopamine release (Zhang and Sulzer, 2004; Rice and Cragg, 2004). Additionally, ethanol has been shown to interact with nAChRs to produce changes in dopamine levels (for review see Hendrickson et al., 2013). Because the inhibitory effects of ethanol and nAChR-modulation of dopamine release are both frequency-dependent, we examined nAChR involvement in the dopamine-decreasing effects of ethanol. C57 and DBA mice express a number of differences in cholinergic systems, including enzymatic activity and nAChR sensitivity to ethanol (Iacopino et al., 1986; Butt et al., 2003; Symons et al., 2010). Therefore, in an attempt to investigate potential mechanisms of ethanol's effects on dopamine release in the two mouse strains, we investigated whether nAChR blockade would reduce ethanol-mediated inhibition of dopamine release under high-frequency conditions. Specifically, we examined the effects of ethanol in the presence of the non-selective nAChR antagonist mecamylamine, the more selective β2-nAChR antagonist dihydro-β-erythroidine hydrobromide (DhβE), and the α6-nAChR antagonist α-conotoxin MII [H9A; L15A] (α-Ctx).

EXPERIMENTAL PROCEDURES

Animals

Male C57BL/6J and DBA/2J mice (Jackson Labs; aged 7–9 weeks) were given *ad libitum* access to food and water, and maintained on a reverse 12:12-h light/dark cycle (lights on at 15:00 h). All protocols and animal care procedures were in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and approved by the Wake Forest School of Medicine Institutional Animal Care and Use Committee. All efforts were made to minimize animal suffering and the number of animals used in the present study.

Brain slice preparation

Isoflurane (Patterson Veterinary, Devens, MA, USA) anesthetized mice were sacrificed by decapitation and brains were rapidly removed and transferred into ice-cold, pre-oxygenated (95% O_2/5% CO_2) artificial cerebral spinal fluid (aCSF) consisting of (in mM): NaCl