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Switch from excitatory to inhibitory actions of ethanol on dopamine levels after chronic exposure: Role of kappa opioid receptors

Anushree N. Karkhanis [a, b], Kimberly N. Huggins [a], Jamie H. Rose [a], Sara R. Jones [a, b, *]

[a] Department of Physiology and Pharmacology, Wake Forest School of Medicine, Winston-Salem, NC, USA
[b] Translational Center for the Neurobehavioral Study of Alcohol, Wake Forest School of Medicine, Winston-Salem, NC, USA

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ABSTRACT

Acute ethanol exposure is known to stimulate the dopamine system; however, chronic exposure has been shown to downregulate the dopamine system. In rodents, chronic intermittent exposure (CIE) to ethanol also increases negative affect during withdrawal, such as, increases in anxiety- and depressive-like behavior. Moreover, CIE exposure results in increased ethanol drinking and preference during withdrawal. Previous literature documents reductions in CIE-induced anxiety-, depressive-like behaviors and ethanol intake in response to kappa opioid receptor (KOR) blockade. KORs are located on presynaptic dopamine terminals in the nucleus accumbens (NAc) and inhibit release, an effect which has been linked to negative affective behaviors. Previous reports show an upregulation in KOR function following extended CIE exposure; however it is not clear whether there is a direct link between KOR upregulation and dopamine downregulation during withdrawal from CIE. This study aimed to examine the effects of KOR modulation on dopamine responses to ethanol of behaving mice exposed to air or ethanol vapor in a repeated intermittent pattern. First, we showed that KORs have a greater response to an agonist after moderate CIE compared to air exposed mice using *ex vivo* fast scan cyclic voltammetry. Second, using *in vivo* microdialysis, we showed that, in contrast to the expected increase in extracellular levels of dopamine following an acute ethanol challenge in air exposed mice, CIE exposed mice exhibited a robust decrease in dopamine levels. Third, we showed that blockade of KORs reversed the aberrant inhibitory dopamine response to ethanol in CIE exposed mice while not affecting the air exposed mice demonstrating that inhibition of KORs "rescued" dopamine responses in CIE exposed mice. Taken together, these findings indicate that augmentation of dynorphin/KOR system activity drives the reduction in stimulated (electrical and ethanol) dopamine release in the NAc. Thus, blockade of KORs is a promising avenue for developing pharmacotherapies for alcoholism.

1. Introduction

Drug addiction is a chronically relapsing disorder that progresses from positive reinforcement, drug induced elevation in mood, to negative reinforcement, wherein drugs are used to alleviate negative emotional states (Koob et al., 2014; Wee and Koob, 2010). Escalation in consumption and repeated cycles of drug intake and withdrawal result in neuroadaptive changes leading to an emergence of negative affect such as anxiety, anhedonia, and depression (Weiss et al., 2001). Both cocaine (Buffalari et al., 2012) and ethanol (Rose et al., 2016) withdrawal has been shown to increase anxiety-like behavior in rodents. Withdrawal-induced increased anxiety in turn results in enhancement of drug seeking behavior to reduce negative affect (Koob, 2013). Repeated exposure to ethanol develops tolerance to many of ethanol's effects, such as sedation (Broadwater et al., 2011), locomotion (Zapata et al., 2006), and social activity (Varlinskaya and Spear, 2007). Thus it is possible that animals increase drug consumption in order to achieve the pre-tolerant positive reinforcing effects and to alleviate negative affective symptoms, leading to greater withdrawal-induced anxiety, and so on. This vicious cycle leads to excessive and compulsive abuse of drugs such as alcohol, leading ultimately to addiction.

Ethanol has multiple pharmacological targets of action in the brain, including the dopamine and dynorphin/kappa opioid receptor (KOR) systems. Acute administration of ethanol results in a

* Corresponding author. Department of Physiology and Pharmacology, Wake Forest School of Medicine, Medical Center Boulevard, Winston-Salem, NC 27157, USA.
E-mail address: srjones@wakehealth.edu (S.R. Jones).

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transient elevation in extracellular accumbal dopamine (Imperato and Di Chiara, 1986; Yim et al., 1998) likely driven primarily by an increased firing rate of dopaminergic neurons in the ventral tegmental area (VTA; Brodie et al., 1999). Conversely, chronic ethanol administration results in a functional decrease in dopamine system function with attenuated firing rates (Bailey et al., 2001; Shen, 2003), reduced electrically stimulated dopamine release, increased uptake rates (Karkhanis et al., 2015; Rose et al., 2016), and attenuated ethanol-evoked dopamine responses (Zapata and Shippenberg, 2006; Zapata et al., 2006). With respect to the dynorphin/KOR system, at high doses acute ethanol exposure increases extracellular levels of dynorphin in the NAc (Marinelli et al., 2006). Recent studies have shown that chronic ethanol exposure enhances KOR responsiveness to agonist (Rose et al., 2016), augments KOR coupling with G-protein (Kissler et al., 2014) and alters dynorphin expression and levels, although the direction and magnitude of change is variable (Lindholm et al., 2001; Kissler et al., 2014; Rose et al., 2016).

Within the NAc, KORs are expressed presynaptically on dopamine terminals and suppress dopamine release when activated (Werling et al., 1988; Svingos et al., 2001; Ebner et al., 2010). The KOR system appears to exert tonic control on dopamine levels in the NAc, because infusion of KOR antagonists and genetic deletion of KORs results in increased basal dopamine levels in the NAc (Spanagel et al., 1992; Chefer et al., 2005). Conversely, acute administration of KOR agonists reduces basal dopamine levels (Spanagel et al., 1990) and opposes the increase in dopamine following administration of drugs of abuse including heroin (Xi et al., 1998), cocaine (Maisonneuve et al., 1994), amphetamine (Gray et al., 1999) and ethanol (Lindholm et al., 2007). It is likely that interactions between dopamine and dynorphin/KOR systems contribute to neurochemical and behavioral changes following chronic intermittent ethanol (CIE) exposure. Thus, determining the impact of KOR system activity on dopamine signaling after CIE offers a potential target for therapeutic treatments of alcohol abuse and dependence.

The goal of these studies was to test the hypothesis that CIE produces an overall attenuation of dopamine responses, and that these changes are mediated, at least in part, by KOR activation. We exposed adult mice to CIE vapor and measured dopamine activity with *in vivo* microdialysis and *ex vivo* voltammetry. We further examined the responsiveness of KORs to agonist using voltammetry to confirm the hypothesis that KOR function was enhanced following moderate (3 cycle) CIE exposure. Additionally, we examined the effect of acute ethanol challenge on dopamine responses in the NAc of air and CIE exposed mice using microdialysis. While there was no difference in tonic baseline levels of dopamine between air and CIE exposed mice, KORs showed increased activity in CIE compared to air exposed mice, and acute ethanol unexpectedly decreased extracellular dopamine levels, an effect that was reversed by a KOR antagonist. These data suggest that chronic ethanol exposure-induced tolerance of dopamine responses to ethanol is potentially driven via elevated KOR signaling. The reversal of ethanol-induced dopamine response following KOR blockade confirm increased dynorphin/KOR system activity further suggests that drugs targeting this system may prove to be promising pharmacotherapies to treat alcoholism.

2. Methods

2.1. Animals

Male C57BL/6 mice (8–12 weeks; Jackson Laboratories, Bar Harbor, ME) were used for all experiments. Animals were housed individually with food and water *ad libitum* (12-hr light-dark cycle). Experimental protocols adhered to National Institutes of Health Animal Care Guidelines and were approved by the Wake Forest School of Medicine Institutional Animal Care and Use Committee.

2.2. CIE and withdrawal

The design of the repeated ethanol exposure and withdrawal paradigm was adapted from Becker and colleagues (Becker, 1994; Becker et al., 1997) with minor modifications. Mice were assigned to either control/air or ethanol exposure groups. The ethanol group underwent 16 h of continuous ethanol vapor exposure followed by 8 h off in room air each day for four days, followed by three days of abstinence (1 cycle of CIE; Fig. 1A), this was repeated three times for a total of 3 cycles of CIE. A loading dose of 1 g/kg ethanol (20% w/v) and the ethanol dehydrogenase inhibitor, pyrazole (85 mg/kg) in 0.9% saline was administered i.p. to the mice each day prior to entering the ethanol vapor inhalation chamber. Following the injections, mice were placed inside the ethanol vapor chamber (within their home cages). Ethanol was delivered to the chamber by volatilizing 190 proof ethanol. The ethanol concentration was maintained by mixing the ethanol vapor with fresh air at a rate of 10 L/min. The control group was treated identically to the ethanol group, with administration of i.p. pyrazole only before they were placed in chambers flowing fresh air. Blood samples were collected and analyzed as described before (Karkhanis et al., 2015). The average blood ethanol concentration for the mice exposed to three cycles of the ethanol vapor was 180 ± 15 mg/dL (mean ± SEM).

2.3. Brain slice preparation

Immediately upon completion of the vapor exposure, 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 (126), KCl (2.5), NaH_2PO_4 (1.2), $CaCl_2$ (2.4), $MgCl_2$ (1.2), $NaHCO_3$ (25), glucose (11), L-ascorbic acid (0.4) and pH was adjusted to 7.4. The brain was sectioned into 400 μm-thick coronal slices containing the striatum with a vibrating tissue slicer (Leica VT1000S, Vashaw Scientific, Norcross, GA) and transferred to a submersion recording chamber perfused at 1 ml/min at 32 °C with oxygenated aCSF.

2.4. Fast scan cyclic voltammetry

Following an equilibration period (30-min), a carbon fiber microelectrode (approximately 150 μM length, 7 μM radius) and a bipolar stimulating electrode were placed in close proximity to each other (approximately 100 μM apart) into the NAc core (Fig. 1B). DA was evoked by a single, rectangular, electrical pulse (300 μA, 2 ms) applied every 5 min. Extracellular DA was recorded every 100 ms using fast-scan cyclic voltammetry (Calipari et al., 2012) by applying a triangular waveform (−0.4 to +1.2 to −0.4 V *vs* Ag/AgCl, 400 V/s). One slice was used per animal (air, $n = 5$; CIE, $n = 5$). After achieving a stable dopamine response, cumulative concentration-response curve was obtained for U69,593 (30, 100, and 300 nM), a KOR agonist, with each dose added after signal stability was reached (approximately 45 min). After dopamine response was stabilized for the final concentration of U69,593, norbinaltorphimine (norBNI; 10 μM), a KOR antagonist, was bath applied to the slices to verify that the effect of U69,593 on dopamine release was in fact due to KOR activation. Immediately following the completion of each experiment, recording electrodes were calibrated by recording their response (in current; nA) to 3 μM dopamine in aCSF using a flow-injection system.

To determine kinetic parameters, evoked levels of dopamine were modeled using Michaelis–Menten kinetics, as a balance