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Inhibition of AMPA receptor and CaMKII activity in the lateral habenula reduces depressive-like behavior and alcohol intake in rats

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ABSTRACT

Depression is a well-known risk factor for developing relapse drinking, but the neuronal mechanisms underlying the interactions between depression and alcohol use disorders remain elusive. Accumulating evidence has associated depression with hyperactivity of the lateral habenula (LHb), an epithalamic structure in the brain that encodes aversive signals. Glutamate receptors contribute substantially to the excitability of LHb neurons. Glutamatergic synapses in LHb neurons largely express GluA1-containing α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors (AMPAR) that can be modulated by Ca^{2+}/calmodulin-dependent protein II (CaMKII). In the current study, we tested the hypothesis that withdrawal from repeated cycles of ethanol drinking triggers an increase in LHb AMPAR and CaMKII activity concomitant with depression-like symptoms, and their inhibitions bring a reduction in depressive-like behaviors and alcohol consumption. Western blotting revealed a higher level of phosphorylated AMPAR GluA1 subunit at a CaMKII locus (GluA1-Ser831) in the LHb of ethanol-withdrawn rats than that of age-matched naïve counterparts. In ethanol-withdrawn rats, pharmacological inhibition of LHb AMPAR activity significantly mitigated the depressive-like behavior and ethanol drinking and seeking behaviors, but affected neither sucrose intake nor locomotor activity; and inhibition of LHb CaMKII activity, or chemogenetic inhibition of LHb activity produced similar effects. Conversely, activation of LHb AMPARs induced depressive-like behaviors in ethanol-naïve rats. These results demonstrate that CaMKII-AMPAR signaling in the LHb exemplifies a molecular basis for depressive-like symptoms during ethanol withdrawal and that inhibition of this signaling pathway may offer a new therapeutic approach to address the comorbidity of alcohol abuse and depression.

1. Introduction

Alcoholics frequently suffer from depression (Pettinati, 2004), which negatively impacts treatment outcomes and increases the probability of relapse (Pelc et al., 2002). The mechanisms underlying this comorbidity, however, are not well understood. Increasing evidence has linked depression with hyperactivity of the lateral habenula (LHb). The LHb has emerged as an important part of the reward circuit by providing negative reward signals to the dopamine and serotonergic neurons in the midbrain (Jhou et al., 2009; Kaufling et al., 2009; Matsumoto and Hikosaka, 2007; Omelchenko et al., 2009). Various aversion-related stimuli, such as stress, pain, fear, and reward omission, activate LHb neurons (Li et al., 2011a; Meye et al., 2015; Neumann et al., 2015; Wirtshafter et al., 1994; Zouikr et al., 2014). LHb neurons are hyperactive in the depressed state (Chourbaji et al., 2005; Proulx et al., 2014; Shumake et al., 2003; Zouikr et al., 2014). Conversely, inhibition of LHb neuronal activity suppresses depressive-like behaviors in animal models of depression (Li et al., 2011a, 2013; Meye et al., 2015). However, the precise molecular targets responsible for LHb hyperactivity during ethanol withdrawal have not been fully unveiled.

Glutamate is the primary excitatory neurotransmitter in the CNS. α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid

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receptors (AMPAR), a subclass of ionotropic glutamate receptors, play an essential role in regulating synaptic strength (Bredt and Nicoll, 2003), and are involved in several neurological, psychiatric and addictive disorders (Zhang and Abdullah, 2013), including alcohol use disorder (Kryger and Wilce, 2010). AMPARs are composed of four subunits (GluA1-4). The GluA1 subunit, in particular, plays an important role in alcohol reinforcement, drinking and seeking behaviors (Cannady et al., 2017; Salling et al., 2016; Wang et al., 2012). The LHb receives strong glutamatergic inputs and expresses mostly the GluA1 subunit (Meye et al., 2013). Enhanced glutamatergic transmission might activate Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII) (Fink and Meyer, 2002), which then phosphorylates serine 831 (S831) on the GluA1 C-terminus, enhancing AMPAR activity (Hayashi et al., 2000; Malinow, 2003). A previous animal study found that increased expression of βCaMKII in the LHb causes more GluA1 insertion into synapses, resulting in increased synaptic efficacy and depressive-like behaviors (Li et al., 2013).

We have recently shown that increased glutamatergic transmission in the LHb contributes to the increased activity of LHb neurons in rats withdrawn from chronic ethanol consumption and that LHb inhibition by high frequency electrical stimulation reduces ethanol consumption (Li et al., 2017). However, it is unknown whether CaMKII-AMPAR signaling in the LHb is functionally relevant for depressive-like behavior and elevated ethanol drinking following a history of repeated cycles of drinking and withdrawal. In the current study, using a combination of behavioral, physiological, pharmacological, molecular, and chemogenetic approaches, we tested the hypothesis that during ethanol withdrawal, the activity of AMPARs and CaMKII in the LHb is increased, which contributes to depressive-like symptoms and elevated alcohol consumption; and that inhibition of the activity of LHb AMPAR and CaMKII ameliorates depressive-like behaviors and reduces alcohol consumption.

2. Materials and methods

2.1. Animals and housing

All experiments were performed in accordance with the guidelines of the National Institutes of Health for the Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committees of Rutgers, The State University of New Jersey, Newark, New Jersey. Adult male Long-Evans rats (two months old at the start of the experiments, from Harlan Lab, NY) were individually housed in ventilated Plexiglas cages in a climate-controlled room (20–22 °C) and acclimatized to the housing conditions and handling for at least 7 days before the start of the experiments. All rats were kept on a 12-h light/dark cycle: lights off at 11:00 a.m. Food and water were available ad libitum, or as indicated otherwise.

2.2. Ethanol drinking procedure

2.2.1. Intermittent access to ethanol in two-bottle free choice(IA2BC) drinking procedure

We trained rats to drink 20% ethanol in the IA2BC procedure as described previously (Li et al., 2011b, 2017; Simms et al., 2008). Briefly, animals were given 24-h concurrent access to one bottle of 20% (v/v) ethanol in water and one bottle of water, starting at 11:00 a.m. on Monday. After 24 h, the ethanol bottle was replaced with a second water bottle that was available for the next 24 h. This pattern was repeated on Wednesdays and Fridays. On all other days, the rats had unlimited access to two bottles of water. On each ethanol-drinking day, the placement of the ethanol bottle was alternated to account for side preferences. The amount of ethanol or water consumed was determined by weighing the bottles before and after 24 h of access. Animal body weight was measured weekly to monitor health and to calculate ethanol intake. Ethanol consumption was determined by calculating grams of alcohol consumed per kilogram of body weight. The preference ratio of ethanol intake was calculated by the following formula: Preference ratio (%) = ethanol solution intake (ml)/total fluid intake (ml ethanol solution + ml water).

After eight-weeks in the IA2BC procedure, when a stable baseline drinking level (5.3 ± 1.2–6.2 ± 0.8 g/kg/24 h) had been achieved, rats were divided into 9 groups (S Table 1): (1) Forced swimming test (G1a, n_{rats} = 12), (2) Sucrose preference test (G1b, n_{rats}=8), (3), Tissues containing the LHb were harvested at 24 h after the last ethanol session for electrophysiological recordings (G1c, n_{rats} = 7), or for examination of AMPAR and CaMKII expression using Western blot (G1d, n_{rats} = 6). Rats in groups 2–7 all received LHb cannulae implantation. Specifically, rats in group 2–5 were examined for the effects of DNQX through: forced swimming test (G2, n_{rats} = 22), sucrose preference test (G3, n_{rats} = 18), ethanol intake (G4, n_{rats} = 9), in operant chamber testing self-administration of ethanol (G5, n_{rats} = 8). Rats in groups 6 and 7 were examined for the effects of CaMKII inhibitor KN-62 through: depressive-like behavior and ethanol intake (G6, n_{rats} = 20), and GluA1 phosphorylation (G7, n_{rats} = 12): rats in this group were sacrificed 10 min after intra-LHb infusion of KN-62 or aCSF, tissues containing the LHb were then collected for Western blot. Rats in group 8 received cannulae implantation to the mediodorsal thalamic nucleus (MD) to examine the effect of DNQX on ethanol intake (n_{rats} = 7). Rats in group 9 received chemogenetic virus injection in the LHb to test whether chemogenetic inhibition of LHb changed ethanol intake and depressive like behaviors (n_{rats} = 12).

2.2.2. Operant self-administration after intermittent access to ethanol

The operant chamber (30 cm wide, 29 cm high) was encased within a larger sound-attenuating chamber and had two levers that were located against the right wall, 7 cm from the floor and 1 cm from the right or left edge of the right wall. A 2.5-cm white stimulus light was located above each lever. A rectangular recess (3 cm in diameter) was located between the 2 levers, 3 cm above the floor. Syringe pumps delivered fluid into a fluid receptacle within this recess (ethanol port). A house light, located on the right wall 14 cm from the floor, was on for the duration of each behavioral session. In addition, the operant chamber contained an infrared head poke detector that recorded how many times an animal's head entered the ethanol reward port. All behavioral equipment (MED Associates, St. Albans, VT) was computer-controlled via software (MED Associates) that also recorded the responses and reinforcer deliveries during behavioral sessions.

This experiment was conducted similarly to our previous studies (Fu et al., 2016; Li et al., 2012). Briefly, a group of rats (n = 8) under the IA2BC paradigm for 16 to 20 sessions received 3 overnight (12–14 h) sessions with 0.1 ml of 20% ethanol available on a fixed-ratio-1 (FR1) schedule after responses to the active lever. After shaping, subjects received daily 45-min sessions, 5 days a week. One week later, the response requirement was increased to a FR3 schedule in 30-min sessions for 2 weeks.

2.3. Brain slice preparation and electrophysiological recordings

Rats at 24 h withdrawal from ethanol after eight weeks in the IA2BC paradigm or ethanol-naïve counterparts were sacrificed under anesthesia of ketamine/xylazine (80 mg/10 mg/kg, i.p). Brain slices were prepared as described (Zuo et al., 2013). Briefly, the