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Reduced alcohol intake and reward associated with impaired endocannabinoid signaling in mice with a deletion of the glutamate transporter GLAST

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ABSTRACT

A hyperglutamatergic state has been hypothesized to drive escalation of alcohol intake. This hypothesis predicts that an impairment of glutamate clearance through inactivation of the astrocytic glutamate transporter, GLAST (EAAT1), will result in escalation of alcohol consumption. Here, we used mice with a deletion of GLAST to test this prediction. WT and GLAST KO mice were tested for alcohol consumption using two-bottle free-choice drinking. Alcohol reward was evaluated using conditioned place preference (CPP). Sensitivity to depressant alcohol effects was tested using the accelerating rotarod, alcohol-induced hypothermia, and loss of righting reflex. Extracellular glutamate was measured using microdialysis, and striatal slice electrophysiology was carried out to examine plasticity of the cortico-striatal pathway as a model system in which adaptations to the constitutive GLAST deletion can be studied. Contrary to our hypothesis, GLAST KO mice showed markedly decreased alcohol consumption, and lacked CPP for alcohol, despite a higher locomotor response to this drug. Alcohol-induced ataxia, hypothermia, and sedation were unaffected. In striatal slices from GLAST KO mice, long-term depression (LTD) induced by high frequency stimulation, or by post-synaptic depolarization combined with the L-type calcium channel activator FPL 64176 was absent. In contrast, normal synaptic depression was observed after application of the cannabinoid 1 (CB1) receptor agonist WIN55,212-2. Constitutive deletion of GLAST unexpectedly results in markedly reduced alcohol consumption and preference, associated with markedly reduced alcohol reward. Endocannabinoid signaling appears to be down-regulated upstream of the CB1 receptor as a result of the GLAST deletion, and is a candidate mechanism behind the reduction of alcohol reward observed.

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1. Introduction

Glutamatergic dysregulation has been hypothesized as a key pathophysiological factor in alcoholism, and a mechanism that could be targeted by pharmacotherapies for this disorder (De Witte et al., 2003; Spanagel, 2009; Spanagel and Kiefer, 2008; Tsai and Coyle, 1998). Brain microdialysis in rats has directly shown an increase in extracellular glutamate levels during withdrawal from alcohol (Dahchour et al., 1998; Rossetti and Carboni, 1995), and has shown that this increase is progressive with consecutive cycles of intoxication and withdrawal (Dahchour and De Witte, 2003). Conversely, glutamate reuptake inhibition using dl-threo-β-benzyloxyaspartic acid (TBOA) has been shown to increase voluntary alcohol consumption (Kapasova and Szumlinski, 2008). Accordingly, acamprosate, a putative functional glutamate antagonist, is an approved medication for the treatment of alcoholism (Bouza et al., 2004; Spanagel and Kiefer, 2008).

Clearance of extracellular glutamate is critical for glutamate homeostasis, and occurs in large part through the activity of the glutamate transporters GLAST (EAAT1) and GLT-1 (EAAT2), which are primarily expressed by astrocytes (Danbolt, 2001; Rothstein et al., 1996). Up-regulation of GLAST gene expression

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accompanies escalation of alcohol consumption in rats following prolonged intermittent brain alcohol exposure (Rimondini et al., 2002), and has also been reported in post-mortem brain tissue from alcoholics (Flatscher-Bader et al., 2006; Flatscher-Bader and Wilce, 2006). In these cases, up-regulation of GLAST can be viewed as a homeostatic adaptation to elevated extracellular glutamate levels caused by repeated cycles of intoxication and withdrawal. Once this mechanism becomes insufficient to maintain glutamate homeostasis, a persistent hyperglutamatergic state can emerge, and continue to drive escalated alcohol consumption. In other cases, deficient GLAST function may be pre-existing due to e.g. genetic factors, and directly drive a hyperglutamatergic state accompanied by escalation of alcohol consumption.

Both these scenarios predict that disruption of GLAST function should lead to escalation of alcohol consumption. Support for this prediction has come from work with mutant mice in which deletion of *Per2* (periodic clock gene 2) was associated with deficient GLAST expression and function (Spanagel et al., 2005). This was accompanied by an increase in extracellular glutamate and escalation of voluntary alcohol consumption, both of which were rescued by acamprosate (Spanagel et al., 2005). In a parallel to these findings, acamprosate, an approved alcoholism treatment, suppressed central glutamate levels in recently detoxified alcoholics as measured by magnetic resonance spectroscopy (MRS) (Umhau et al., 2010).

The *Per2* study found that alcohol consumption was inversely correlated with GLAST function, but did not establish a causal relationship between the two. Strong evidence for causality, and support for the hyperglutamatergic theory of alcoholism would be obtained if disruption of GLAST function were found to result in escalation of alcohol consumption. This would also offer an attractive *in vivo* model to evaluate novel candidate medications that target the glutamatergic system. Here, we examined this possibility. Pharmacological tools to modulate GLAST function with a high degree of specificity are lacking; for instance, the reuptake inhibitor TBOA does not distinguish between the EAATs. Here, we therefore used mice with a genetic deletion of GLAST (Watase et al., 1998), and evaluated them for alcohol consumption and reward, as well as sensitivity to depressant effects of alcohol. In search of the mechanistic underpinnings of our behavioral findings, we then performed microdialysis for glutamate in the Nc. Accumbens, a key structure for drug reward. Synaptic plasticity in the cortico-striatal pathway was finally assessed because it offers a well characterized model of glutamate dependent plasticity, in which potential adaptations to the constitutive GLAST deletion can be studied. Because endocannabinoid transmission is an established modulator of both glutamatergic transmission and alcohol consumption, its integrity was examined in this model system.

2. Materials and methods

2.1. Animals

Generation of the GLAST knock-out (KO) mice has been described (Watase et al., 1998). The GLAST gene was disrupted in embryonic stem (ES) cells from 129/Sv mice, and replaced by a neomycin resistance cassette in exon 6. The targeted ES clone was injected into C57BL/6J blastocysts to create chimeric mice, and mice were backcrossed onto a C57 background for >10 generations. In order to avoid a potential confound from genotypic differences in maternal behavior and early life environment (Millstein and Holmes, 2007), GLAST KO, GLAST HET and WT mice were all generated from HET × HET mating.

Mice were housed in groups of 1–4 per cage, in a temperature and humidity controlled vivarium, under 12-h light/dark cycle, and with *ad libitum* access to food and water in the home cage. Males and females at least 8 weeks of age were tested, and the experimenter remained blind to the genotype during testing. All experimental procedures were approved by the National Institute of Alcohol Abuse and Alcoholism Animal Care and Use Committee and followed the NIH guidelines 'Using Animals in Intramural Research'; or were approved by the Committee on Animal Care and Use (Regierungspräsidium Karlsruhe), and carried out in accordance with the local Animal Welfare Act and the European Communities Council Directives.

2.2. Two-bottle free-choice alcohol consumption

Mice were single caged, habituated to a new animal room for two weeks, and then given continuous free access to increasing concentrations of an aqueous alcohol solution or water. For the first 7 days, access was given to two bottles containing tap water, and intake was examined for possible side preference. Alcohol was then provided in one of the bottles and faded in (2%, vol/vol; 4–5 days, 4%, 4–5 days), after which consumption was measured at 8%, 12%, and 16%, obtaining 6 data points at each concentration over the course of 7–8 days. The amount of alcohol ingested was expressed as g/24 h/kg body weight.

2.3. Conditioned place preference for alcohol

CPP for alcohol was examined as previously described (Boyce-Rustay and Cunningham, 2004). Time spent and locomotor activity within each compartment were measured by photobeams placed 1.2 cm apart across the full length of the apparatus. The grid and mesh floors were covered with solid Plexiglas to prevent conditioning to the floor texture. Mice were first given a habituation session in which they received an i.p. saline injection and were allowed to freely explore the apparatus for 5 min. Twenty-four hours later, conditioning started, using an unbiased design in which half of the subjects received 2 g/kg alcohol (CS+) or saline (CS−), and were placed into the appropriate compartment for 5 min. CS+ and CS− trials were alternated daily, and their order was counterbalanced within genotype. One complete trial consisted of a CS+ trial and a CS− trial. After 6 conditioning trials, preference was tested by giving the animal a saline injection, and placing it into the center compartment to freely explore the whole apparatus for 10 min.

2.4. Fear conditioning

Fear conditioning was assessed as previously described (Karlsson et al., 2005; Kim and Fanselow, 1992). Briefly, the Freeze Monitor system (San Diego Instruments, San Diego, CA, USA) was used, and automatically delivered a 30 s 80 dB auditory conditioned stimulus (CS) and a 0.6 mA 2 s footshock unconditioned stimulus (US). Twenty-four hours after conditioning, mice were placed in a novel context, allowed to habituate for 180 s, and then presented with the auditory CS and evaluated for freezing for 180 s. Additional 24 h later, freezing induced by re-exposure to the training context was tested for 300 s. The presence of freezing behavior was scored every 10 s, and was defined as the absence of any movement except respiration. Data were calculated as the proportion of observations in which the subject was scored as freezing.

2.5. Sensitivity to depressant effects of alcohol

Depressant effects of alcohol could interfere with place conditioning, and could also be aversive. To examine possible genotype differences in the sensitivity to depressant alcohol actions, we assessed alcohol-induced ataxia, hypothermia and sedation/hypnosis. A single cohort of mice was tested in all three assays. Because the respective behaviors are sensitive to different doses of alcohol, testing started with the assay involving the lowest dose (i.e., ataxia), followed by hypothermia, and last sedation/hypnosis. At least 1 week was allowed between tests. This regimen has been employed in previous studies (Boyce Rustay et al., 2006; Boyce-Rustay and Holmes, 2006) and is not thought to produce lasting tolerance to alcohol (Crabbe et al., 2008).

To assess alcohol-induced ataxia, mice were placed onto the rotarod dowel, which was accelerated at a constant rate of 8 rpm/min up to 40 rpm. The latency to fall off was recorded by photocell beams, with a maximum cutoff latency of 5 min. Mice received 10 training trials separated by a 30-sec inter-trial interval. Twenty-four hours later, there was a baseline habituation trial, followed by 2 baseline trials that were averaged to obtain a single measure of pre-alcohol treatment performance. Mice were then injected with 2 or 2.25 g/kg alcohol. Thirty min thereafter, they were given an additional habituation trial, followed by 2 test trials that were averaged to obtain a single measure of post-alcohol treatment performance. The dependent measure was the difference between pre- and post-alcohol treatment performance (=delta latency).

Basal core body temperature was measured by inserting a Thermalert TH-5 thermometer (Physitemp, Clifton, NJ, USA) 2 cm into the rectum until a stable reading was obtained. Mice were then injected with 3.0 or 4.0 g/kg alcohol, and temperature was measured 30, 60, 90, and 120 min later to provide an average post-alcohol measure. The difference between pre-alcohol versus post-alcohol treatment temperature was the dependent measure (=delta temperature). Ambient room temperature was 23 °C.

To assess alcohol-induced sedation, loss of righting reflex (LORR) was studied. Mice were injected with 3.0 or 4.0 g/kg alcohol and placed into the supine position in a V-shaped chamber. The time from injection to recovery of the righting reflex (turning onto all 4 paws twice in 30 s after initial self-righting) was measured.