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Chronic voluntary alcohol consumption results in tolerance to sedative/hypnotic and hypothermic effects of alcohol in hybrid mice

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ABSTRACT

The continuous two-bottle choice test is the most common measure of alcohol consumption but there is remarkably little information about the development of tolerance or dependence with this procedure. We showed that C57BL/6J×FVB/NJ and FVB/NJ×C57BL/6JF1 hybrid mice demonstrate greater preference for and consumption of alcohol than either parental strain. In order to test the ability of this genetic model of high alcohol consumption to produce neuroadaptation, we examined development of alcohol tolerance and dependence after chronic self-administration using a continuous access two-bottle choice paradigm. Ethanol-experienced mice stably consumed about 16–18 g/kg/day of ethanol. Ethanol-induced withdrawal severity was assessed (after 59 days of drinking) by scoring handling-induced convulsions; withdrawal severity was minimal and did not differ between ethanol-experienced and -naïve mice. After 71 days of drinking, the rate of ethanol clearance was similar for ethanol-experienced and -naïve mice. After 77 days of drinking, ethanol-induced loss of righting reflex (LORR) was tested daily for 5 days. Ethanol-experienced mice had a shorter duration of LORR. For both ethanol-experienced and -naïve mice, blood ethanol concentrations taken at gain of righting reflex were greater on day 5 than on day 1, indicative of tolerance. After 98 days of drinking, ethanol-induced hypothermia was assessed daily for 3 days. Both ethanol-experienced and -naïve mice developed rapid and chronic tolerance to ethanol-induced hypothermia, with significant group differences on the first day of testing. In summary, chronic, high levels of alcohol consumption in F1 hybrid mice produced rapid and chronic tolerance to both the sedative/hypnotic and hypothermic effects of ethanol; additionally, a small degree of metabolic tolerance developed. The development of tolerance supports the validity of using this model of high alcohol consumption in genetic studies of alcoholism.

1. Introduction

Alcohol abuse is a prerequisite for development of alcoholism and is associated with a high cost to health and quality of life. In 2004, the US Department of Health estimated that 22.5 million Americans have experienced substance abuse or dependence (Chou and Narasimhan, 2005). According to the Diagnostic and Statistical Manual-IV, dependence on alcohol is accompanied by signs of abuse, compulsive drinking behavior, tolerance, and withdrawal, all of which promote alcohol intake and increase the damaging effects of alcohol over time. Development of alcohol tolerance requires that more alcohol is needed to achieve an effect; therefore the effect of a given dose of ethanol decreases as tolerance develops. Three categories of ethanol tolerance have been described according to the sequential order in which they appear. Acute-functional tolerance occurs when an initial response to ethanol is reduced within a single drinking session in a metabolism-independent manner (Mellanby, 1919). Acute functional tolerance occurs within a time frame lasting minutes to hours. Rapid tolerance occurs 8 to 24 h after a single ethanol administration (Crabbe et al., 1979; Rustay and Crabbe, 2004). Chronic tolerance occurs after repeated ethanol administration and can last for days, months, or years. Chronic tolerance can be environment-dependent or -independent. Characterization of rapid and chronic tolerance has been described for repeated trials of ethanol-induced hypothermia and loss of righting reflex (LORR; Crabbe, 1989, 1994; Crowell et al., 1981; Lê et al., 1979; Melchior and Tabakoff, 1981; Silvers et al., 2003). Human and animal studies indicate that some aspects of tolerance are genetically determined; thus tolerance has an innate component (Browman et al., 2000; Crabbe, 1994; Crabbe et al., 1994; Garcia-Andrade et al., 1997; Phillips et al., 1996; Schuckit and Gold, 1988; Schuckit et al., 2004).

Alcohol dependence is also characterized by the presence of withdrawal symptoms (physical and psychological) after drinking ceases, which results from physical dependence on alcohol. Withdrawal from alcohol in humans is characterized by central nervous system hyperexcitability, seizures, autonomic dysregulation, anxiety, restlessness, nausea, sleeplessness, and depression. Nearly all studies of

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ethanol withdrawal in mice focus on one or two behaviors — seizure severity and anxiety-like behavior. In rodent models, dependence on ethanol is frequently induced either by offering an ethanol-containing liquid diet as the sole source of nutrition or by ethanol vapor inhalation. Once dependence is induced, withdrawal seizure severity is measured using handling-induced convulsion (HIC) scores (Goldstein and Pal, 1971). Increased HIC scores can be seen after a single injection of ethanol using a modification of the HIC index, allowing the detection of withdrawal severity with more sensitivity (Crabbe et al., 1991). This behavioral index ranges from facial grimace to tonic–clonic convulsions. The severity of withdrawal depends on genetic background, as well as dose of ethanol and duration of exposure (Crabbe et al., 1991; Goldstein and Pal, 1971; Metten et al., 1998). For a more detailed review of alcohol tolerance and dependence, several excellent reviews are available (Harris and Buck, 1990; Kalant et al., 1971; Kalant, 1998; Koob and Bloom, 1988; Phillips et al., 1994).

The use of rodent models to model human disease has been a powerful tool in the advancement of understanding disease and improving treatments. There are several rodent models in place to study alcohol-related phenotypes. We found that C57BL/6J×FVB/NJ (B6xFVB) and FVB/NJ×C57BL/6J (FVBxB6) F1 hybrid mice self-administer unusually large amounts of alcohol during two-bottle preference tests (females consume 20–35 g/kg/day, males 7–25 g/kg/day, depending on concentration) (Blednov et al., 2005). The distinction between the hybrids is discerned by the order of the listed cross, where the first strain listed is the dam and the second strain of the cross is the sire. This new genetic model has significant advantages when compared to existing inbred strains, including evidence of drinking to intoxication (Blednov et al., 2005). Using this mouse model of high alcohol consumption, we studied tolerance and withdrawal following chronic alcohol consumption. To our knowledge, this is the first study to evaluate the acute withdrawal, metabolic, sedative/hypnotic, and hypothermic responses to ethanol after chronic self-administration using a continuous access two-bottle choice paradigm. Two sets of chronic ethanol drinking experiments were carried out using the two-bottle choice paradigm. The first set of experiments consisted of chronic ethanol drinking followed by testing for physical dependence (ethanol-induced acute withdrawal), rapid and chronic hypothermic tolerance, and rapid sedative/hypnotic tolerance. The second set of experiments consisted of chronic ethanol drinking followed by testing for metabolic tolerance (ethanol clearance) and rapid and chronic sedative/hypnotic tolerance. We found that chronic, high levels of alcohol consumption in F1 hybrid mice produced rapid and chronic tolerance to both the sedative/hypnotic and hypothermic effects of ethanol; additionally, a small degree of metabolic tolerance developed. The development of tolerance supports the validity of using this model of high alcohol consumption in genetic studies of alcoholism.

2. Methods

2.1. Animals

Studies were conducted using intercross F1 hybrid female mice derived from C57BL/6J and FVB/NJ mice (B6xFVB F1 and FVBxB6 F1). C57BL/6J and FVB/NJ breeders were purchased from The Jackson Laboratory (Bar Harbor, ME) and mated at 7–8 weeks. Mice were housed individually in standard cages. Behavioral testing did not begin until the mice were at least 70 days old. Ethanol-naïve and ethanol-experienced groups were closely matched for age, and littermates were split between the two groups. Except for one occasion in the first set of experiments, all mice were tested only once. Ethanol-naïve and -experienced mice were tested for acute ethanol withdrawal and retested approximately one month later for ethanol-induced loss of righting reflex. Experiments were conducted in the isolated behavioral testing rooms in the animal facility to avoid external distractions. The University of Texas facility is accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care (AAALAC). All experiments were approved by the Institutional Animal Care and Use Committee and adhered to NIH Guidelines.

2.2. Drugs

Ethanol was obtained from Aaper (Shelbyville, KT) and was dissolved in 0.9% saline and injected intraperitoneally (i.p.) in a volume of 0.2 ml per 10 g of body weight.

2.3. Continuous access two-bottle choice

For ethanol-naïve groups, water was available ad libitum throughout the study. For ethanol-experienced groups, water and an ethanol solution were presented in identical bottles and continuously available in the two-bottle choice paradigm. Bottle positions were changed daily for both ethanol-experienced and -naïve groups. Mice were weighed every 8 days throughout the experiment. Initially, the mice were offered water and 3% ethanol (v/v in tap water) for 4 days, and then escalating concentrations (3% increases up to 18%, then 20% ethanol thereafter) were offered versus water for 4 days each. Initially, fluid consumption was measured daily, found to be stable and therefore, only measured every other day (beginning eight days following introduction of the 20% ethanol solution). FVBxB6 female mice were used for the first set of experiments (Fig. 1a, ethanol-experienced group, n = 23 total). B6xFVB female mice were used for the repeated LORR experiment in the second set of experiments (Fig. 1b, ethanol-experienced group, n = 9). FVBxB6 female mice were used for the clearance experiment in the second set of experiments (Fig. 1c, ethanol-experienced group, n = 4). Fig. 1b and c represents a second set of experiments carried out at the same time. Refer to Fig. 1 for details on the timing of behavioral testing and the genotypes used for each test. Furthermore, ethanol, but not water, was removed several hours before behavioral testing. Behavioral testing occurred during mid-day (during lights on and presumably when lower levels of ethanol were consumed in the prior hours). Mice were acclimated to the behavioral rooms one hour prior to loss of righting reflex and ethanol clearance testing. Whereas, hypothermia and handling-induced convulsion testing was measured from home cages.

2.4. Ethanol-induced acute withdrawal

After 59 days of two-bottle choice, ethanol-experienced (n = 11) and ethanol-naïve (n = 10) mice were scored for handling-induced convulsion (HIC) severity 30 min before ethanol administration (3.8 g/kg). The HIC score was tested every hour until the HIC level reached baseline. Each mouse was picked up gently by the tail and, if necessary, gently rotated 180°, and the HIC was scored as follows: 5, tonic–clonic convulsion when lifted; 4, tonic convulsion when lifted; 3, tonic–clonic convulsion after a gentle spin; 2, no convulsion when lifted, but tonic convulsion elicited by a gentle spin; 1, facial grimace only after a gentle spin; and 0, no convulsion after a gentle spin (18).

2.5. Ethanol clearance

After 71 days of two-bottle choice, ethanol-experienced (n = 4) and ethanol-naïve (n = 4) mice received ethanol (4 g/kg), and rates of ethanol clearance were determined using a spectrophotometric enzyme assay. Blood samples (50 μL) were taken from the retro-orbital sinus (at 30, 60, 120, 180, and 240 min post-injection), added to 2 mL 3% perchloric acid, and centrifuged for 10 min at 1000×*g*. Resulting supernatants were used to determine blood ethanol concentration using an ethanol dehydrogenase enzyme assay (Lundquist, 1959).