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Role of interleukin-1 receptor signaling in the behavioral effects of ethanol and benzodiazepines

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ABSTRACT

Gene expression studies identified the interleukin-1 receptor type I (IL-1R1) as part of a pathway associated with a genetic predisposition to high alcohol consumption, and lack of the endogenous IL-1 receptor antagonist (IL-1ra) strongly reduced ethanol intake in mice. Here, we compared ethanol-mediated behaviors in mice lacking *Il1rn* or *Il1r1*. Deletion of *Il1rn* (the gene encoding IL-1ra) increases sensitivity to the sedative/hypnotic effects of ethanol and flurazepam and reduces severity of acute ethanol withdrawal. Conversely, deletion of *Il1r1* (the gene encoding the IL-1 receptor type I, IL-1R1) reduces sensitivity to the sedative effects of ethanol and flurazepam and increases the severity of acute ethanol withdrawal. The sedative effects of ketamine and pentobarbital were not altered in the knockout (KO) strains. Ethanol intake and preference were not changed in mice lacking *Il1r1* in three different tests of ethanol consumption. Recovery from ethanol-induced motor incoordination was only altered in female mice lacking *Il1r1*. Mice lacking *Il1rn* (but not *Il1r1*) showed increased ethanol clearance and decreased ethanol-induced conditioned taste aversion. The increased ethanol- and flurazepam-induced sedation in *Il1rn* KO mice was decreased by administration of IL-1ra (Kineret), and pre-treatment with Kineret also restored the severity of acute ethanol withdrawal. Ethanol-induced sedation and withdrawal severity were changed in opposite directions in the null mutants, indicating that these responses are likely regulated by IL-1R1 signaling, whereas ethanol intake and preference do not appear to be solely regulated by this pathway.

1. Introduction

There is substantial evidence that alcohol exposure activates neuroimmune signaling and that these pathways participate in many acute and chronic actions of alcohol (Crews et al., 2011; Mayfield et al., 2013). Much of the initial interest in neuroimmune molecules came from gene expression studies in human alcoholics or from rodents with a genetic predisposition for high alcohol consumption, showing an unexpected differential expression of immune genes in the brain (Liu et al., 2004; Mulligan et al., 2006). Subsequent studies investigated the role of these genes in alcohol actions. For example, ethanol consumption in null mutant mice lacking specific immune-related genes [beta-2-microglobulin (*B2m*), cathepsin S (*Ctss*), cathepsin F (*Ctsf*), interleukin-1 receptor antagonist (IL-1ra) (*Il1rn*), CD14 molecule (*Cd14*), interleukin-6 (*Il6*)] showed that deletion of any one of these genes decreased ethanol intake and preference (Blednov et al., 2012).

Other studies also demonstrated a role for IL-1 receptor type I (IL-1R1) signaling in alcohol responses. The human recombinant IL-1R1 antagonist (IL-1ra, Kineret) reduced alcohol sedation and motor impairment (Wu et al., 2011), and Kineret reduced alcoholic steatohepatitis in mice (Petrasek et al., 2012). Chronic alcohol consumption increased IL-1β in brain (Lippai et al., 2013), and genetic polymorphisms in *IL1B* and *IL1RN* were associated with risk for alcohol dependence in humans (Liu et al., 2009; Pastor et al., 2005).

The IL-1R1 signaling system (consisting of the cytokines IL-1α or IL-1β, IL-1R1, and the endogenous antagonist IL-1ra) is activated peripherally by infection and inflammation and is also important in brain function (Arend and Guthridge, 2000). IL-1β enhances GABAergic and glycinergic function in brain (Brambilla et al., 2007; Chirila et al., 2014; Serantes et al., 2006), and allelic variants of genes for IL-1β are associated with depression (Bufalino et al., 2013). Although activation of immune function is proposed to

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increase ethanol consumption (Blednov et al., 2011), mice lacking *Il1rn* (i.e., deficient in IL-1ra) showed markedly decreased ethanol consumption in three different tests (Blednov et al., 2012). These results prompted us to employ a combination of genetic and pharmacological approaches to define the role of IL-1R1 signaling in the behavioral actions of ethanol, benzodiazepines, and other sedative drugs. We used mice lacking *Il1r1* (the gene encoding the IL-1 receptor type I, IL-1R1) or mice lacking *Il1rn* (the gene encoding the receptor antagonist, IL-1ra) to inhibit and enhance IL-1R1 signaling, respectively, and Kineret, an injectable form of IL-1ra, as a pharmacological manipulation.

2. Materials and methods

2.1. Animals

Il1r1 [B6.129S7-*Il1r1*^{tm1Imx}/J (Stock #003245)] or *Il1rn* [B6.129S-*Il1rn*^{tm1Dih}/J (Stock #004754)] KO mice were purchased from Jackson Laboratories (Bar Harbor, ME). *Il1rn* heterozygous KO mice were purchased, and the colony was maintained by heterozygous breeding. *Il1r1* homozygous KO mice were purchased and bred with C57BL/6J to produce heterozygous mice, and the colony was maintained by heterozygous breeding. Wild type (WT) mice from the same colonies were used as controls. *Il1r1* KO mice are referred to as *Il1r* in the figures and figure legends. Two different 129 sub-strains were used in the generation of the KO mice and *Il1r1* KO mice were then bred on a C57BL/6J background (a high alcohol drinking strain), which may account for the different ethanol responses that we observed in WT mice from the two colonies. However, all KO mice were analyzed with their WT counterparts, thus controlling for differences in genetic background between WT and corresponding KO mice. After weaning, mice were housed in the Animal Resources Center at The University of Texas at Austin in rooms with 12-h light/dark cycles (lights on at 7:00 a.m.) with *ad libitum* access to rodent chow and water. Male and female mice 8–12 weeks of age were used. In some experiments, data from males and females were combined if there were no sex differences, and in other experiments, only male mice were tested (see Results and Figure legends). Each mouse was used for only one experiment, and all mice were ethanol naive at the start of each test. Experiments were approved by the Institutional Animal Care and Use Committee at The University of Texas (#AUP 2013-00061) and were conducted in accordance with the National Institutes of Health guidelines with regard to the care and use of animals in research. Recent studies demonstrate that behavioral results are strongly influenced by the experimenter (Bohlen et al., 2014; Sorge et al., 2014). To minimize potential "investigator" effects, experiments were carried out by the same three investigators, with each investigator being responsible for two or three different experiments.

2.2. Ethanol drinking-24-h access

A two-bottle choice protocol was carried out as previously described (Blednov et al., 2003). Mice were allowed to acclimate for 1 week to individual housing. Two drinking bottles were continuously available to each mouse, and bottles were weighed daily. One bottle always contained water. Food was available *ad libitum*, and mice were weighed every 4 days. After 4 days of water consumption (both bottles), mice were offered 3% ethanol (v/v) vs. water for 4 days. Bottle positions were changed daily to control for position preferences. Quantity of ethanol consumed (g/kg body weight/24 h) was calculated for each mouse, and these values were averaged for every concentration of ethanol. Immediately following 3% ethanol, a choice between 6% (v/v) ethanol and water was offered for 4 days, followed by 9, 12, 15, and finally 18% (v/v) ethanol (each concentration was offered for 4 days). Throughout the experiment, evaporation/spillage estimates were calculated daily from 2 bottles placed in an empty cage; one bottle contained water, and the other contained the appropriate ethanol solution.

When measuring the effects of Kineret on ethanol intake, baseline drinking was first established. WT and *Il1rn* KO male mice consumed 10% ethanol for at least 3 weeks. After this period, ethanol consumption was measured for at least 4 days to ensure stable consumption. The criterion for stable consumption was similar values for days 1–2 and days 3–4. For the 24-h two-bottle choice test, ethanol intake was then measured after saline injection for 2 days, and mice were grouped to provide similar levels of ethanol intake and preference. Ethanol and total fluid intake were presented as g/kg/24 h. Saline or Kineret (100 mg/kg) was administered i.p. 30 min before placement of the bottles.

2.3. Ethanol drinking-limited access drinking in the dark (one-bottle DID)

Consumption of ethanol (20% solution) under conditions of limited access achieves pharmacologically significant levels of ethanol drinking (Rhodes et al., 2005). Starting 3 h after lights off, the water bottles were replaced with bottles containing a 20% ethanol solution. The ethanol bottles remained in place for either 2 (days 1–3) or 4 h (day 4) and then were replaced with water bottles. Other than these short periods of ethanol drinking, mice had unlimited access to water. The ethanol bottles were weighed before placement and after removal from the experimental cages.

2.4. Ethanol drinking-limited access drinking in the dark (two-bottle choice DID)

This was similar to the one-bottle DID test described above except that 2 bottles containing 20% ethanol vs. water were used. The ethanol and water bottles remained in place for 3 h. After their removal, mice had unlimited access to 1 bottle of water. The positions of bottles during 3-h access were changed daily to avoid potential side preferences. The ethanol and water bottles were weighed before placement and after removal from the experimental cages.

2.5. Conditioned taste aversion (CTA)

Mice were adapted to a water-restriction schedule (2 h of water per day) over a 7-day period. At 48-h intervals ondays 1, 3, 5, 7, 9 and 11, all mice received 1-h access to a solution of saccharin (0.15% w/v sodium saccharin in tap water). Immediately after 1-h access to saccharin, mice received injections of saline or 2.5 g/kg ethanol (days 1, 3, 5, 7 and 9). Mice also received 30-min access to water 5 h after each saccharin-access period to prevent dehydration (days 1, 3, 5, 7 and 9). On intervening days, mice had 2-h continuous access to water at standard times in the morning (days 2, 4, 6, 8 and 10). Reduced consumption of the saccharin solution is used as a measure of CTA.

2.6. Ethanol-induced acute withdrawal

Mice were scored for handling-induced convulsion (HIC) severity 30 min before and immediately before i.p. ethanol administration. These two pre-drug baseline scores were averaged. A dose of 4.0 g/kg of ethanol in saline was injected i.p., and the HIC score was tested every hour until the HIC level reached baseline. Acute withdrawal was quantified as the area under the curve that was above the pre-drug level (Crabbe et al., 1991). Each mouse is picked up gently by the tail and, if necessary, gently rotated 180°, and the HIC is 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; 0, no convulsion.

2.7. Loss of righting reflex (LORR)

Sensitivity to the depressant effects of ethanol (3.4 and 3.6 g/kg), flurazepam (225 mg/kg), pentobarbital (50 mg/kg), and ketamine (175 mg/kg) were measured using the standard duration of LORR (sleep time) test in mice. When mice became ataxic, they were placed in the supine position in V-shaped plastic troughs until they were able to right themselves 3 times within 30 s. Sleep time was defined as the time from being placed in the supine position until they regained their righting reflex. An ethanol dose of 3.4 g/kg was used when measuring the effects of Kineret on the duration of LORR.

2.8. Rotarod

Mice were trained on a fixed speed rotarod (Economex; Columbus Instruments, Columbus, OH) at 5 rpm, and training was complete when mice were able to remain on the rotarod for 60 s. Every 15 min after injection of ethanol (2.0 g/kg i.p.), each mouse was placed on the rotarod and latency to fall was measured until the mouse was able to stay on the rotarod for 60 s.

2.9. Elevated plus maze

Mice were evaluated for basal anxiety as well as ethanol-induced anxiolysis using the elevated plus maze as described previously (Blednov et al., 2001). Mice were transported to the testing room 1 day before testing and were tested between 10:00 and 12:00 a.m. under ambient room light. Mice were weighed and injected with ethanol (1.25 g/kg, i.p.) or saline 10 min before testing. Each mouse was placed on the central platform of the maze facing an open arm. Mice were allowed to freely explore the maze for 5 min during which the following measurements were manually recorded: number of open arm entries, number of closed arm entries, total number of entries, time spent in open arms, and time spent in closed arms. The mouse was considered to be on the central platform or any arm when all four paws were within its perimeter.

2.10. Ethanol clearance

Animals were given a single dose of ethanol (4.0 g/kg, i.p.), and blood samples were taken from the retro-orbital sinus 30, 60, 120, 180, and 240 min after injection. Blood ethanol concentration (BEC) values were determined spectrophotometrically by an enzymatic assay (Lundquist, 1959).

2.11. Rationale for the behavioral tests

Two-bottle choice (continuous, 24-h access) is the most widely used test of ethanol preference and voluntary consumption and is related to other measures of ethanol reward (Green and Grahame, 2008). Other tests of ethanol intake (one- and two-bottle DID) produce high levels of ethanol consumption by limiting access to