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Decreased anxiety, voluntary ethanol intake and ethanol-induced CPP acquisition following activation of the metabotropic glutamate receptor 8 "mGluR8"

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ABSTRACT

Metabotropic glutamate receptors (mGluRs) are important modulators of excitatory neurotransmission, and have been implicated in addiction to alcohol and anxiety-related behaviors. However, the behavioral consequence and contribution of individual subtypes are not known yet. This study determined the effects of mGluR8 activation on anxiety-like behavior, voluntary ethanol intake and ethanol-induced conditioned reward. To this aim, anxiety and spontaneous behavior were measured in C57BL/6J mice using the elevated plus maze (EPM), open field (OF) and light-dark box (LDB) tests after systemic injection of the selective mGluR8 agonist (*S*)-3,4-dicarboxyphenylglycine ((*S*)-3,4-DCPG). In addition, the anti-alcohol properties of mGluR8 were studied using a two-bottle choice continuous access drinking paradigm and ethanol-conditioned place preference (CPP). Results have shown that, compared to vehicle, DCPG produced an anxiolytic-like effect in the LDB, and OF tests. Furthermore, DCPG-injected mice displayed significantly lower intake and preference for ethanol [2.5–20% (v/v) escalating over 2 weeks] in a two-bottle choice paradigm, with no significant difference observed with saccharin [0.04 & 0.08% (w/v)] nor on quinine [20 & 40 μM (w/v)]. Interestingly, DCPG administration attenuated ethanol-induced acquisition, but not expression, of CPP. More importantly, these effects were significantly attenuated when mice were pre-injected with the group III mGluR-specific antagonist (*S*)-2-amino-2-methyl-4-phosphonobutyric (MAP4). These data demonstrate that activation of the mGluR8 reduces voluntary ethanol intake in male mice and eliminates place preference acquisition suggesting that mGluR8 signaling may contribute to the rewarding properties of ethanol. Taken together, these findings demonstrate that mGluR8-targeted pharmacotherapies may be beneficial for the treatment of anxiety and alcoholism.

1. Introduction

Alcoholism is one of the leading causes of preventable death worldwide and chronic exposure to ethanol results in long-lasting neuroadaptations in multiple signaling pathways, including opioid, dopamine, glutamate, and GABA pathways (for review, see Chastain, 2006; Heinz et al., 2009). These long-lasting changes increase the difficulty of achieving long-term abstinence. Nevertheless, it has been proposed that metabotropic glutamate receptors (mGluRs) may be the prime candidates key players for mediating this vulnerability to alcoholism (Holmes et al., 2013; Lavreysen and Dautzenberg, 2008).

Glutamate is the major excitatory neurotransmitter in the central nervous system (CNS) and its transmission plays a key role in alcohol addiction. Glutamate activates eight different mGlu receptors that have been identified to date and classified based on sequence homology, pharmacology and coupling to intracellular effectors. The mGluR family, which are G-protein coupled receptors, was clustered into three groups: Group I (mGluR1 and mGluR5), Group II (mGluR2 and mGluR3) and Group III (mGluR4, mGluR6, mGluR7, and mGluR8) (for review, see Conn and Pin, 1997). The group III family is the least studied of the three groups due to the lack of suitable selective pharmacological agents. Nevertheless, this group of receptors are beginning to emerge as important contributors to stress-related disorders such as depression, anxiety, addiction and schizophrenia (Goudet et al., 2008; Lavreysen and Dautzenberg, 2008). Therefore, a large amount of evidence suggests that targeting the mGluR system may be helpful in the treatment of addiction disorders in general and alcoholism in particular (Koob and Volkow, 2010).

Abbreviations: CPP, Conditioned Place Preference;; DCPG, (*S*)-3,4-dicarboxyphenylglycine; EPM, Elevated Plus Maze; EtOH, Ethanol; LDB, Light-Dark Box; MAP4, (*S*)-2-Amino-2-methyl-4-phosphonobutyric; mGluR, Metabotropic Glutamate Receptors; OF, Open Field.

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The mGluR8 is expressed in the thalamus, the globus pallidus, the nucleus accumbens (NAcc), substancia nigra (SN), the striatum, the subthalamic nuclei, *etc.* (Messenger et al., 2002). The anatomical distribution of mGluR8 mRNA at a large range of neuroanatomical regions known to be associated with number of psychiatric and neurological disorders suggests that this receptor could be a potential therapeutic target. In fact, mGluR8 has been implicated in the pathophysiology of number of disorders including anxiety (Duvoisin et al., 2010; Duvoisin et al., 2005), stress-induced pathologies (O'Connor et al., 2013) and schizophrenia (Robbins et al., 2007; Takaki et al., 2004). More importantly, when Parelkar and co-workers examined alterations in mGluR8 mRNA expression in the rat forebrain in response to repeated intraperitoneal administration of amphetamine using quantitative *in situ* hybridization they found that mGluR8 mRNA levels were profoundly increased in the dorsal (caudate putamen) and ventral (NAcc) striatum as well as in the cerebral cortex (Parelkar and Wang, 2008) suggesting that this receptor subtype might play an important role in addiction.

A number of studies have examined the effects of glutamatergic neurotransmission using elevated plus maze (EPM), the open field (OF), and the light–dark box (LDB) tests, which are paradigms of unconditioned conflict anxiety that model generalized anxiety disorders (GAD) (Zimmer et al., 2015). To extend our previous studies on the implication of group III mGluRs in psychiatric disorders, we have utilized the selective mGluR8 agonist DCPG, to examine its effects on anxiety-like behavior using the EPM, OF, and LDB tests. Based on our previous studies on mGluR7 (Bahi, 2012, 2013c; Bahi et al., 2012), we hypothesized that vehicle- and DCPG-injected mice would display differences in their ethanol-drinking behavior. In the present study, we examined the role of mGluR8 activation, using DCPG, in a two-bottle choice paradigm, to measure voluntary ethanol intake and preference. We thus hypothesized that mGluR8 activation would alter alcohol-drinking behavior and that treatment with MAP4 would abrogate that behavior. Furthermore, the present study examined mGluR8 activation in an ethanol-induced conditioned place preference (CPP) paradigm. In this procedure, we measured the animals' tendency to approach or avoid environmental cues previously paired with the drug and several studies have demonstrated that as with other drugs of abuse, animals display CPP to ethanol (for review see Cunningham et al., 2003). Therefore, another objective of the present study was to determine if the mGluR8 is important in Pavlovian conditioning to the ethanol-paired environmental cues. We hypothesized that mGluR8 activation would attenuate the acquisition but not the expression of ethanol CPP.

2. Materials & methods

2.1. Animals

Adult C57BL/6J male mice (20 to 30 g) were obtained from the central breeding facility of the College of Medicine & Health Sciences. Animals were housed in groups of five per cage under environmentally controlled conditions (ambient temperature, approximately 22 °C; humidity, 50–60%) and were given 10 days for acclimation to a 12:12-h light/dark cycle (light on between 6:00 am and 6:00 pm) with food and water available *ad libitum*. The standard rodents chow diet was obtained from the National Feed and Flour Production and Marketing Company LLC (Abu Dhabi, UAE). All experiments were executed in accordance with the Guide for the Care and Use of Laboratory Animals and were approved by the local Animal Research and Ethics Committee (Approval No A09-11).

2.2. Drugs

For the two-bottle choice drinking procedure, ethanol solutions (2.5, 5, 10 and 20%, v/v) were prepared from absolute ethyl alcohol (Panreac Quimica SAU, Barcelona, Spain) and diluted using tap water. For taste sensitivity, saccharin sodium salt dihydrate (0.04 and 0.08%; w/v) and quinine hemisulfate (20 and 40 μM; w/v) were purchased from Sigma-Aldrich (St. Louis, MO, USA) and were dissolved in tap water. For the CPP experiments, ethanol (10% *v/v*) was dissolved in physiological saline 0.9% sodium chloride and sterile water and used for intraperitoneal (i.p.) injections at dose of 2 g/kg. This dose was based on previous studies from our laboratory showing ethanol CPP with C57BL/6 mice (Bahi, 2013a, 2012; Bahi and Dreyer, 2014, 2012b; Bahi et al., 2013a; Bahi et al., 2013b). Diazepam 1 mg/kg manufactured by Gulf Pharmaceutical Industries (Ras Al Khaimah, United Arab Emirates) was obtained from Dr. Essam Emam (Department of Medicine, Tawam Hospital, Al Ain, United Arab Emirates). The mGluR8 agonist (*S*)-3,4-dicarboxyphenylglycine ((*S*)-3,4-DCPG) and the group III mGluR antagonist (*S*)-2-Amino-2-methyl-4-phosphonobutyric (MAPs4, 0.3 mg/kg) were dissolved in 0.9% sodium chloride and sterile water and administered intraperitoneally at a volume of 10 mL/kg once a day 30 min before the light goes off. Doses of DCPG and MAP4 were selected based on previous published reports. In wild-type mice, DCPG (100 mg/kg) significantly increased c-Fos expression in several stress-related brain regions. However, c-Fos expression was unchanged by DCPG in mGlu8 receptor knockout mice (Linden et al., 2003). Also, DCPG (30 mg/kg, i.p.) reduced seizure activity in rats (Robbins et al., 2007). For the group III mGluR antagonist MAP4, it has been shown that the effects of the selective mGluR8 agonist (DCPG) and the effects of the mGluR7 agonist (AMN082) on evoked EPSCs and synaptic spiking were blocked by the group III antagonist MAP4 (Ren et al., 2011).

2.3. Anxiety-like behavior

2.3.1. Elevated plus maze (EPM)

The EPM test was performed out as originally described by (Pellow et al., 1985) and as carried out in our laboratory in multiple studies (Bahi, 2013b; Bahi et al., 2016; Bahi et al., 2014a; Bahi and Dreyer, 2014, 2012a; Bahi et al., 2014b). In brief, the EPM apparatus consisted of two open arms (OA) (40×6 cm^2), which are facing two opposite closed arms (CA) ($40 \times 6 \times 20$ cm^3) connected by a central platform (6×6 cm^2) elevated 50 cm from the floor. Each mouse was placed at the center of the maze, with the head facing an open arm. The number of entries and the time spent in both OA and CA were live hand scored during a 5-min period by an experienced observer. An entry was scored as such only when the animal placed all four limbs into any given arm. The dose-related effects of DCPG (100 mg kg^{-1}, i.p.) and a comparison with that produced by diazepam (1 mg kg^{-1}, i.p.) were assessed.

2.3.2. Open field (OF) test

The OF test was performed as previously described for C57BL/6 mice with few modifications (Bahi, 2013a, b; Bahi and Dreyer, 2012a). The apparatus consisted of a square ($100 \times 100 \times 20$ cm^3) arena. The floor was marked into 64 equal squares and the central 16 squares were defined as the center area. A 60 W light bulb was positioned 100 cm above the base of the arena. Each mouse was individually placed in the center of the arena for 5 min and the time spent in the center of the arena (indicative of decreased anxiety) together with the number of squares crossed (indicative of spontaneous locomotor activity) were hand scored by the experimenter. Square crossing was scored only when the hind limbs of the animal moved to the next square. At the end of each test the number of fecal boli was counted and the arena was cleaned with 70% ethyl alcohol and dried between trials.

2.3.3. Light dark box (LDB) test

The original method described by (Crawley and Goodwin, 1980) was used with slight modifications. In brief, the apparatus consisted of two boxes ($24 \times 30 \times 30$ cm^3). The light chambers had a fixed 60 W bulb as its light source located 30 cm above the box. The boxes were connected by a 6-cm high guillotine door that allowed the mice to move freely between the two chambers. Mice were released into the upper corner of the light chamber and was keenly observed for latency