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A transcriptional study in mice with different ethanol-drinking profiles: Possible involvement of the $GABA_B$ receptor

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ABSTRACT

Previous studies have suggested that γ-aminobutyric acid-B ($GABA_B$) receptor agonists effectively reduce ethanol intake. The quantification using real-time polymerase chain reaction of *Gabbr1* and *Gabbr2* mRNA from the prefrontal cortex, hypothalamus, hippocampus, and striatum in mice exposed to an animal model of the addiction developed in our laboratory was performed to evaluate the involvement of the $GABA_B$ receptor in ethanol consumption. We used outbred, Swiss mice exposed to a three-bottle free-choice model (water, 5% v/v ethanol, and 10% v/v ethanol) that consisted of four phases: acquisition (AC), withdrawal (W), reexposure (RE), and quinine-adulteration (AD). Based on individual ethanol intake, the mice were classified into three groups: "addicted" (A group; preference for ethanol and persistent consumption during all phases), "heavy" (H group; preference for ethanol and a reduction in ethanol intake in the AD phase compared to AC phase), and "light" (L group; preference for water during all phases). In the prefrontal cortex in the A group, we found high *Gabbr1* and *Gabbr2* transcription levels, with significantly higher *Gabbr1* transcription levels compared with the C (ethanol-naive control mice), L, and H groups. In the hippocampus in the A group, *Gabbr2* mRNA levels were significantly lower compared with the C, L, and H groups. In the striatum, we found a significant increase in *Gabbr1* transcription levels compared with the C, L, and H groups. No differences in *Gabbr1* or *Gabbr2* transcription levels were observed in the hypothalamus among groups. In summary, *Gabbr1* and *Gabbr2* transcription levels were altered in cerebral areas related to drug taking only in mice behaviorally classified as "addicted" drinkers, suggesting that these genes may contribute to high and persistent ethanol consumption.

1. Introduction

Central γ-aminobutyric acid (GABA) neurotransmission is a sensitive target for both the acute and chronic effects of ethanol (Lovinger, 2008). Although several studies have investigated the neuroadaptations associated with ionotropic $GABA_A$ receptors after ethanol use (for review, see Enoch, 2008), the neuroadaptations of metabotropic $GABA_B$ receptors need to be clarified.

Human studies have revealed the efficacy of baclofen (β-parachlorophenol GABA, a $GABA_B$ receptor agonist) in reducing ethanol intake and the compulsive desire for ethanol in dependent individuals (Addolorato et al., 2002; Flannery et al., 2004), but no difference was found in the efficacy of baclofen in other clinical study (Garbutt et al., 2010) although recently Muzyk et al. (2012) reported higher rates of abstinence and lower anxiety scores in baclofen-treated patients.

A reduction in ethanol consumption was observed in Sardinian alcohol-preferring (sP) rats after acute baclofen administration (Maccioni et al., 2005). Baclofen also suppressed the ethanol deprivation effect in rats exposed to ethanol for 8 weeks (Colombo et al., 2003a, 2003b). C57BL/6J mice exhibited increased ethanol consumption after repeated baclofen administration (Moore et al., 2007), although baclofen microinjection into the anterior ventral tegmental area reduced binge-like ethanol intake in the same strain (Moore and Boehm, 2009).

In summary, baclofen, a $GABA_B$ agonist, reduces ethanol intake in animals and humans, but the contrary or no effect was also reported.

Some authors have demonstrated that conformational alteration of the $GABA_{B1}$ subunit and subsequently conformational alteration of the entire $GABA_{B1}$–$GABA_{B2}$ complex is necessary for effective activation of the $GABA_B$ receptor (Morishita et al., 1990). Consequently, the precise balance between the two subunits is necessary for the

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activation of the receptor. One question is how this disproportional receptor subtype expression can support a basis for research on individualizing treatment.

A human study showed no significant association between *GABBR1* gene polymorphisms and alcoholism (Köhnke et al., 2006). However, another analysis suggested a possible association between *GABBR1* and some groups of alcoholics (Sander et al., 1999). Recently, a significant association between *GABBR1* and *GABBR2* and nicotine dependence was demonstrated in an American sample, suggesting a possible correlation between these genes and addictive behavior (Li et al., 2009).

The $GABA_B$ receptor comprises two protein subunits, GB1 and GB2, encoded by the *Gabbr1* and *Gabbr2* genes, respectively. Although the *Gabbr1* gene has various isoforms, the most widely expressed isoforms in the central nervous system are *Gabbr1a* and *Gabbr1b*, which are differentiated by the presence of a sequence that codifies two sushi domains (short consensus repeats, SCR or CPs) in the GB1 protein (Kaupmann et al., 1997; Hawrot et al., 1998).

The $GABA_B$ receptor can be found as a heterodimer (GB1a/GB2 or GB1b/GB2), and both subunits are essential for $GABA_B$ function (Margeta-Mitrovic et al., 2001; Misgeld et al., 1995; Bowery et al., 2002; Bettler et al., 2004; Jones et al., 1998; Chen and van den Pol, 1998; Filippov et al., 2000). Presynaptically, the $GABA_B$ receptor inhibits dopaminergic, GABAergic, and glutamatergic systems, and alterations in these systems are well known to be associated with addictive behavior (for review, see Koob et al., 1998; Everitt and Robbins, 2005; Kalivas and Volkow, 2005; Le Moal and Koob, 2007).

To understand the influence of genes on ethanol intake, some rodent models that use genetic selection have been used and have contributed to a better comprehension of alcoholism, but their results have been inconclusive (Green and Grahame, 2008). A question that arises from the interpretation of studies that involve selective breeding or inbreeding is whether high-drinking lines exhibit greater ethanol-reinforced behavior than low-drinking lines. Moreover, some animal studies may lack many aspects of human alcoholism, such as compulsive drug use, which is characteristic of addiction and central to the clinical diagnosis of dependence (American Psychiatric Association, 1994). Addiction is defined as compulsive drug use despite negative consequences. In recent years, new animal models have been developed and proposed for the study of compulsive drug use (i.e., craving or persistent desire for drug), relapse, and loss of control, which are specific components of human addiction (Heyman, 2000; Phillips, 2002; Shippenberg and Koob, 2002; Spanagel, 2003; Camarini et al., 2010).

The animal model used in the present study was proposed initially for rats by Wolffgramm and Heyne (1995) and validated for mice in our laboratory (Fachin-Scheit et al., 2006). We previously demonstrated the model's reliability, face validity (long-term high ethanol intake and ethanol preference over 4 months, considering the whole life of a mouse, and persistent intake despite bitter taste adulteration of ethanol solutions), and predictive validity (when tested with naltrexone as a pharmacological challenge, mice reduced ethanol intake (Fachin-Scheit et al., 2006; Ribeiro et al., 2008; Correia et al., 2009). The behavioral analysis of fluid intake in mice exposed to a free-choice model was accomplished, and two phenotypes exhibited high ethanol consumption and preference for ethanol during almost the entire treatment (i.e., great behavioral similarity). During the last 2 weeks of the model when ethanol was adulterated with quinine, some high-drinker mice significantly reduced their ethanol intake, whereas others continued to show the same consumption. The "loss of control over the ingestion of ethanol" suggestive of "addiction" (Spanagel, 2009) can be examined in this model only for some mice when ethanol solutions are made "less palatable" by the addition of a quinine solution.

Drug addiction behavior involves different components of the neuronal circuitry like the prefrontal cortex (related with the reinstatement of drug seeking), hippocampus (recognition of contextual conditioned stimuli associated to the drug), hypothalamus (neuroendocrine control), and striatum (related to the drug's rewarding effects). Each of these areas plays a different role in functions related to addictive behavior (for review, see Kalivas and Volkow, 2005; Everitt and Robbins, 2005), with different transcription and protein profiles.

Considering (*i*) the participation of $GABA_B$ receptors in ethanol consumption; (*ii*) the reduction in ethanol consumption under baclofen treatment; (*iii*) the increase in Gabbr2 protein in nicotine addiction; (*iv*) the differential participation of brain structures in the neurobiology of drug-taking behavior; and (*v*) our validated ethanol consumption model provides an alternative approach to the study of addictive behaviors and to the individual ethanol intake profiles, we hypothesized that "addicted" mice might have an increase in Gabbr1 and Gabbr2 transcription levels in those brain areas related to addictive behavior when compared to non-addicted mice (heavy-drinking and light-drinking mice). Thus, the increased GABAB activity would lead to greater inhibition in dopamine release in nucleus accumbens, increasing the rewarding value of ethanol for those mice due to the increased ethanol-induced dopamine release by other mechanisms.

2. Materials and methods

2.1. Animals

Eighty naive 6-week-old Swiss male mice that weighed 20–30 g from the Universidade Federal do Paraná were used in this study. The mice were housed individually (20×30×20 cm) under a 12 h/12 h light/dark cycle (lights on at 07:00 h) and controlled temperature (22 °C ± 2 °C), with ad libitum access to food (Purina Laboratories, Curitiba, Brazil). One week before the treatments, the mice underwent an acclimation period, and water intake and body weight were measured. All procedures were performed during the light cycle. Experimental care and treatment were approved by the Ethics Committee for Animal Experimentation of the Setor de Ciências Biológicas (Protocol Number: 281), Universidade Federal do Paraná.

2.2. Experimental design

2.2.1. Extended chronic ethanol intake

One group of mice ($n = 60$) was exposed to three-bottle free-choice treatment for 10 weeks (acquisition [AC] phase), during which they had free access to both 10% and 5% (v/v) ethanol and water. Another group of control animals ($n = 20$) only had access to water during all phases of the experiment. The experimental design is summarized in Fig. 1. The positions of the bottles were changed on alternate days when fluid intake was measured volumetrically. Over the next 2 weeks, only water was provided [withdrawal (W) phase]. For the following 2 weeks, the ethanol solutions were again offered to establish free-choice responding among the ethanol solutions and water [reexposure (RE) phase]. At the end of this period, the ethanol solutions were adulterated with 0.005 g/L quinine, which produces a bitter-tasting solution, and offered to the animals for a further 2 week period [adulteration (AD) phase]. This quinine concentration was chosen through an analysis of dose–response using other mice, in which the 0.005 g/L concentration of quinine significantly reduced its intake compared with water intake without completely inhibiting response (Fachin-Scheit et al., 2006), suggesting an "aversive-like" effect. This dose–response analyze was based on daily administration of different quinine concentration to different groups of mice (n = 10 per concentration) during seven days. The water and quinine solution consumptions (mL) were performed after 24 h (Table 1).

At the end of the exposition to the 3-bottle free choice paradigm, the mice were classified into groups based on their individual