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Research report

Cellular prion protein (PrP^C) modulates ethanol-induced behavioral adaptive changes in mice

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HIGHLIGHTS

- Genetic deletion of PrP^C decreases ethanol consumption at higher concentrations.
- PrP^C is pivotal to rapid tolerance acquisition.
- D_1-receptor blockade disrupts the abnormal ethanol consumption in PrP^C-KO mice.
- PrP^C is important to D_1 receptor desensitization after chronic exposure to ethanol.

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ABSTRACT

Chronic consumption of drugs with addictive potential induces profound synaptic changes in the dopaminergic mesocorticolimbic pathway that underlie the long-term behavioral alterations seen in addicted subjects. Thus, exploring modulation systems of dopaminergic function may reveal novel targets to interfere with drug addiction. We recently showed that cellular prion protein (PrP^C) affects the homeostasis of the dopaminergic system by interfering with dopamine synthesis, content, receptor density and signaling pathways in different brain areas. Here we report that the genetic deletion of PrP^C modulates ethanol (EtOH)-induced behavioral alterations including the maintenance of drug seeking, voluntary consumption and the development of EtOH tolerance, all pivotal steps in drug addiction. Notably, these behavioral changes were accompanied by a significant depletion of dopamine levels in the prefrontal cortex and reduced dopamine D_1 receptors in PrP^C knockout mice. Furthermore, the pharmacological blockade of dopamine D_1 receptors, but not D_2 receptors, attenuated the abnormal EtOH consumption in PrP^C knockout mice. Altogether, these findings provide new evidence that the PrP^C/dopamine interaction plays a pivotal role in EtOH addictive properties in mice.

1. Introduction

Cellular prion protein (PrP^C) is a highly conserved protein with a sparse distribution in the brain [1]. The presence of this protein at presynaptic and postsynaptic sites [2] is coherent with the evidence that PrP^C-null mice display altered long-term potentiation [3–5]. Although the physiological role of PrP^C remains to be elucidated [6], neuroplasticity-related events such as aging, learning

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and memory and brain development are affected by manipulations of PrP^C [7–9]. Considering the general agreement that neuroplasticity plays a pivotal role in the development of drug addiction [10], it is somewhat surprising that the role of PrP^C on chronic effects of drugs with addictive potential such as ethanol (EtOH) has not been investigated so far and the only study on that matter is based in an acute evaluation [11].

Chronic consumption of drugs with addictive potential, such as EtOH, induces profound synaptic changes of the mesocorticolimbic pathway that underlie the long-term behavioral alterations seen in addicted individuals [12]. This pathway is composed by many brain structures such as the prefrontal cortex, striatum and hippocampus. Dopamine plays a pivotal role in such pathways and, accordingly, the dopaminergic system plays a prominent role in addiction [13]. In particular, EtOH increases the firing rate of dopaminergic neurons in these brain regions, where neuroadaptative structural and functional alterations related to modulation of signaling systems, gene transcription, and protein expression take place during addiction [14]. More recently, we showed that the *Prnp* genetic deletion influences dopamine synthesis, dopamine receptors density and intracellular signaling in different brain regions [15].

The actions of dopamine are mediated through two main classes of receptor subtypes termed D_1-like receptors (D_1 and D_5) and D_2-like receptors (D_2, D_3 and D_4), that induce opposite intracellular responses: D_1 receptors stimulating and D_2 receptors inhibiting cAMP formation [16]. Both subtypes of dopamine receptors have been associated to rewarding, reinforcement and motivational aspects of EtOH consumption [17,18]. In particular, the blockade of dopamine D_1 receptors reduces EtOH consumption [17], and increases the reinforcing properties of EtOH in mice [19]. Moreover, EtOH can modify the dopaminergic tonus by altering the number of dopamine transporter (DAT) expressed at the cell surface, with a direct impact on drug-addiction process [20,21]. In keeping with the major relevance of the dopaminergic mesocorticolimbic pathways for addiction, and the above-mentioned influence of PrP^C on the dopaminergic system, the aim of the present study was to investigate whether PrP^C may impact on the development of EtOH addictive properties in mice using genetic modified mice combined with behavioral and neurochemical assays.

2. Experimental procedures

2.1. Animals

Female knockout mice homozygous for a disruption of the *Prnp* gene, *Prnp* null mice (designated as $Prnp^{0/0}$ mice), produced as previously described [22] and wild-type ($Prnp^{+/+}$) mice were donated by Dr. Vilma R. Martins (International Research Center, A.C. Camargo Hospital) when they were 2–3 months old. The animals were maintained in the animal house of the Universidade Federal de Santa Catarina (Florianópolis, Brazil) until the age of 3–4 months when the experimental protocol started. All animals used in this study weighted 20–30 g. The $Prnp^{0/0}$ mice used were descendants of Zrch I animals, while the wild-type ($Prnp^{+/+}$) controls were generated by crossing F_1 descendants from a 129/Sv × C_{57}BL/6J mating. The genotype of the animals was confirmed by polymerase chain reaction (PCR) with DNA extracted from the tail, using the following primers: forward (5′-ATCAGTCATCATGGCGAAC-3′) and reverse (5′-AGAGAATTCTCAGCTGGATCTTCTCCCGTC-3′). A band of 693 bp corresponds to the *Prnp* sequence in the wild-type animals, while a band of 1635 bp represents the neomycin cassette, which replaced the *Prnp* sequence and thus identifies $Prnp^{0/0}$ mice. Thus, albeit $Prnp^{0/0}$ and $Prnp^{+/+}$ mice were raised and housed side-by-side, it should be stressed that they are not littermates; therefore, the breeding scheme used in this study does not allow excluding the selection of compensatory mechanisms.

All rodents were kept four to a cage and were subjected to a 12-h light/dark cycle (lights on at 7:00 a.m.) with free access to food and water. All procedures used in the present study were approved by the local Ethics Committee on the Use of Animals (CEUA/UFSC PP 00452), which follows the NIH publication "Principles of Laboratory Animal Care".

2.2. Locomotor activity

The effects of EtOH on locomotor activity of mice were investigated in the open field test. The open field apparatus was a 50 cm × 50 cm × 50 cm chamber illuminated to 12 lx. Before the test all animals were weighted. For testing, mice were placed in the center of the open field and their activity recorded during 10 min. The total distance was automatically quantified by the Any-maze® video-tracking system (Stoelting Inc., Kiel, WI, USA). Mice were injected by intraperitoneal (i.p.) route with EtOH (2 g/kg) (analytical grade, VETEC, RJ, Brazil) 10 min before the test during 1, 7, 14 and 21 consecutive days.

2.3. Blood EtOH assay

Ten minutes after the EtOH (2 g/kg, i.p.) administration, blood samples were collected from the animals by direct tail puncture. Blood EtOH concentration was evaluated enzymatically based on EtOH conversion to acetaldehyde by the action of alcohol dehydrogenase [23].

2.4. EtOH voluntary consumption

All mice were isolated and exposed to two inverted bottles with metal sippers placed on stainless steel cage tops during 24 h. Food was distributed near both bottles to avoid food-associated tube preferences. One bottle always contained tap water and the other contained increasing concentration of EtOH (3%, 6%, 10% and 20% v/v in tap water). Mice were exposed to each EtOH concentration for a block of 5 days, according to a previously described procedure [20]. The position of the two bottles was reversed daily during the testing period to prevent position bias. Bottles were weighted daily and EtOH consumption was expressed as grams of EtOH per kilogram of body weight.

2.5. EtOH voluntary consumption (5 h protocol)

Mice were isolated and offered tap water and a 10% (v/v in tap water) EtOH solution in a two-tube free-choice paradigm, using a limited-access model. Fluid intake was monitored during the testing period of 5 h per day during 5 consecutive days to provide an index of consumption by weighting the bottles at the end of the 5 h period [17]. EtOH consumption was expressed as grams of EtOH per kg of body weight for the absolute amount consumed over the 5 h testing period. The position of the two bottles was reversed daily during the testing period to prevent position bias. To test the impact of dopamine receptors, the mice were treated 30 min before the test with the dopamine D_1 receptor antagonist (SCH-23390, 0.1 mg/kg, i.p.) or the dopamine D_2 receptor antagonist (sulpiride, 80 mg/kg, i.p.).

2.6. Rapid tolerance to EtOH-induced motor impairment

The rapid tolerance to EtOH was measured as previously described [24] utilizing the rotarod apparatus (Rotamex-V-EE/85) controlled by a computer system (Columbus Instruments Computer; Columbus, OH, USA). The mice were trained under