Dopamine D3 receptor-dependent changes in GABAA receptor alpha 6 subunit expression control voluntary ethanol intake

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The mesolimbic dopamine (DA) pathway mediates the rewarding effects of drugs of abuse (Wise and Bozarth, 1987) including ethanol, but the role of specific DA receptor subtypes is still unclear. DA exerts its action through five G protein-coupled receptor subtypes (D1–5R); the dopamine D3 receptor (D3R) subtype is involved in the control of several DA-related disorders such as addiction and drug-seeking behavior. We, previously, reported that either D3R gene deletion or D3R pharmacological blockade inhibit ethanol preference and voluntary intake in mice (Leggio et al., 2014). We also reported that the genetic deletion or the pharmacological blockade of D3R affect GABAA receptor subunit expression. In particular, D3R deficient mice (D3R-/-) exhibit a 15-fold higher basal GABAA receptor alpha 6 subunit expression in the ventral striatum compared to their wild-type (WT) littermates (Leggio et al., 2015).

Here we tested the hypothesis that the alpha 6 subunit is involved in ethanol preference and voluntary intake, and that changes in alpha 6 subunit expression induced by D3R deletion or antagonism interfere with the control of ethanol consumption.

D3R-/-, D3R+/- and their WT littermate mice (males, 8-12 weeks old), treated or not with RO 15-4513 (5 mg/kg, intraperitoneally), an agonist of alpha 6 subunit-containing GABAA receptors, were tested in a binge-like ethanol-drinking paradigm, the drinking in the dark (DID). We also analyzed the effects of RO 15-4513 in WT littermates treated with SB-277011A (10 mg/kg, intraperitoneally), a D3R selective antagonist. The alpha 6 subunit mRNA expression was evaluated by Real Time PCR and by in situ hybridization in the ventral striatum, a brain region involved in drug addiction.

The treatment with RO 15-4513 inhibited ethanol intake in WT (day1, Vehicle 4 g/kg; RO 15-45133 2.9 g/kg, p=NS; day 2, Vehicle 5.3 g/kg; RO 15-4513 3.7 g/kg, $p<0.05$; day 3, Vehicle 4.9 g/kg, RO 15-4513 3.4 g/kg, $p<0.05$; day 4, Vehicle 7.72 g/kg, RO 15-4513 7.11 g/kg, p=NS); conversely, it induced an increase in ethanol consumption in D3R-/- (day1, Vehicle 2.37 g/kg; RO 15-4513 3.64 g/kg, p=NS; day 2, Vehicle 2.9 g/kg; RO 15-4513 3.88 g/kg, p=NS; day 3, Vehicle 2.38 g/kg; RO 15-4513 4.41 g/kg, $p<0.05$; day 4, Vehicle 5.07 g/kg; RO 15-4513 7.88 g/kg, $p<0.01$). Consistent with our previous observation, pharmacological blockade of D3R by SB-277011A (repeated treatment for 7 days) inhibited ethanol intake in WT; such an effect was reversed by RO 15-4513.

Analysis of mRNA expression by qPCR revealed an increase of GABAA alpha 6 subunit in WT mice treated with RO 15-4513 in the ventral striatum, but not in D3R-/- mice. In situ hybridization using an anti-sense DNA oligonucleotide probe complementary to alpha 6 GABAA subunit mRNA sequence confirmed the increased expression of alpha 6, particularly in the nucleus accumbens.

In conclusion, changes in alpha 6 GABAA subunit expression control the rewarding properties of alcohol in mice; these changes are strongly influenced by dopamine D3R activity. Thus, the crosstalk between DA and GABA signaling is critically involved in ethanol-related reward and consumption and may provide novel therapeutic strategies for ethanol addiction.

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Wise RA, Bozarth MA (1987). Psychol Rev 94: 469–492.

Leggio GM et al., (2014). Neuropsychopharmacology. 2014 Jul;39(8):2017-28.

Leggio GM et al., (2015). Eur Neuropsychopharmacol. 2015 Sep;25(9):1427-36.