MOLECULAR BASIS OF ALCOHOL INTAKE: ROLE OF D_3 DOPAMINERGIC RECEPTOR

Chiara B. M. Platania [1], Gian Marco Leggio [1], Giovanni Camilleri [1], Alessandro Castorina [2], Giuseppina Marrazzo [1], Sebastiano A. Torrisi [1], Christina N. Nona [3], Velia D'Agata [2], Josè Nobrega [4], Holger Stark [6], Claudio Bucolo [1], Bernard Le Foll [5], Filippo Drago [1] and Salvatore Salomone [1]

1. Department of Clinical and Molecular Biomedicine, Section of Pharmacology and Biochemistry, Catania University. **2.** Department of Bio-medical Sciences, Catania University. **3.** Behavioral Neurobiology Laboratory, Centre for Addiction and Mental Health and Department of Pharmacology and Toxicology. **4.** Imaging Research Centre and Campbell Family Research Institute, Centre for Addiction and Mental Health and Departments of Psychiatry, Psychology and Pharmacology. **5.** Translational Addiction Research Laboratory, Centre for Addiction and Mental Health and Departments of Family and Community Medicine, Psychiatry and Pharmacology, University of Toronto. **6.** Institute for Pharmaceutical and Medicinal Chemistry, Heinrich-Heine-University Düsseldorf.

BACKGROUNG:

The mesolimbic dopamine (DA) pathway mediates the rewarding effects of drugs of abuse, including ethanol and opiates. Dopamine exerts its action through five receptor subtypes ($D_{1\text{-}5}R$); the D_3 receptor (D_3R) subtype plays an important role in the modulation of the mesolimbic DA pathway and in the control of drug-seeking behavior. Several studies have explored the involvement of D_3R in ethanol-drinking paradigms [1]. Here we tested the hypothesis that D_3R gene deletion or the D_3R pharmacological blockade counteracts ethanol preference in mice.

Recently the crystal structure of human D_3 receptor has been solved and the structure models of D_3, D_2 [2] and $5HT_{1A}$ [3] have been optimized and validated by our group. In order to identify the structural basis of antagonism and partial agonism respectively at D_3, D_2 and $5HT_{1A}$ receptors we carried out molecular docking of compounds used in the experimental ethanol-drinking paradigm.

METHODS:

Animals and alcohol drinking paradigms:

Mice D_3R null ($D_3R^{-/-}$) and WT littermates (males, 8–12 weeks old) were individually housed.

-In two-bottle choice paradigm, mice $D_3R^{-/-}$ (n=30) and WT (n=30) received 24 h free access to tap water and 10% ethanol solution (v/v), contained in 100 ml graduated tubes with stainless steel drinking spouts.

-In the drinking in the dark paradigm, the 4 hour version of the behavioral paradigm was used.

Molecular modeling:

Homology modeling of wild-type receptors were obtained using SwissModel (http://swissmodel.expasy.org/) and GPCRRD (http://zhanglab.ccmb.med.umich.edu/GPCRRD/) web servers. Molecular dynamics of receptors was carried out with NAMD 2.8 in water-membrane environment . Molecular docking of compounds was carried out with AutoDock 4.2. Re-scoring of poses was carried out with DSX-Score.

FIG 1

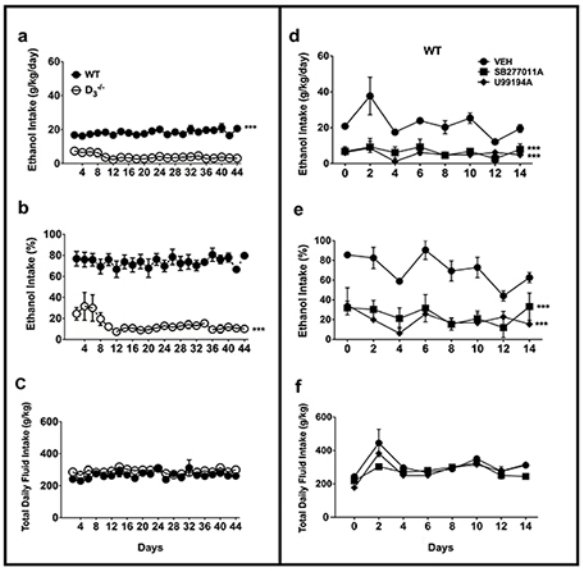


FIG 2

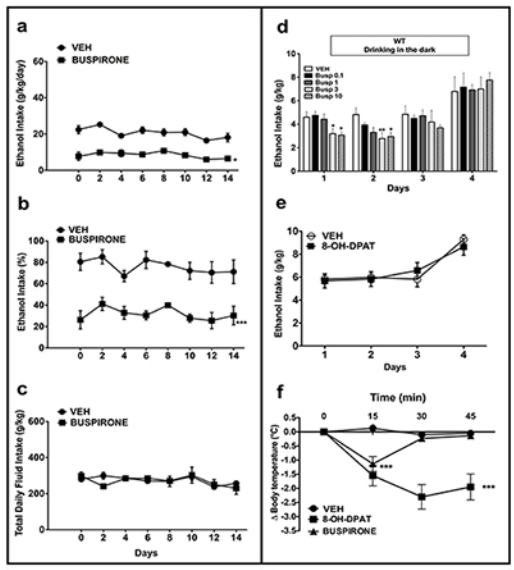


Fig 1.
Either D_3 gene deletion or D_3 pharmacological blockade counteracted the ethanol preference intake and voluntary intake in mice.
D3 -/- and wt littermates, treated or not with D3R selective antagonists were tested in a long term free choice ethanol drinking paradigm (two bottle choice).

Fig 2.
In the two-bottle choice paradigm mice were treated with buspirone (1 mg/kg/day). Treatment of WT mice with buspirone significantly decreased voluntary ethanol intake ($p<0.05$). The treatment with buspirone also significantly decreased ethanol intake in WT mice when tested in the in binge-like ethanol drinking paradigm (Drinking in the Dark DID). DID. The D_3R specific effect of buspirone in decreasing ethanol intake was confirmed by using the selective 5-HT_{1A} agonist, 8-OH-DPAT, that did not affect the fluid ethanol intake. As expected, the 5-HT_{1A} selective agonist 8-OH-DPAT decreased the body temperature of WT mice ($p<0.001$). Only a high dose buspirone (3 mg/kg) decreased the body temperature of WT mice and did so only transiently.

FIG 3. Binding of SB277011A (A) and Buspirone (B) at hD_3 receptor model

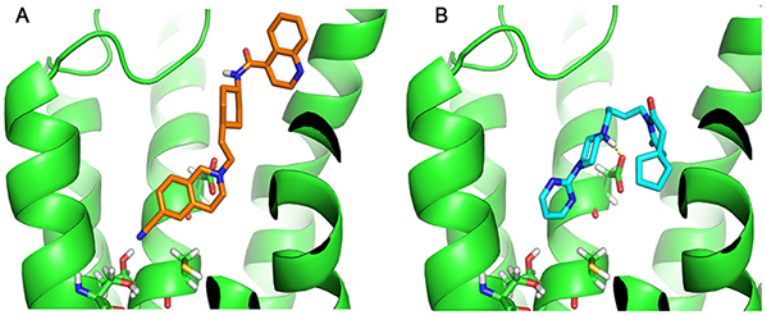
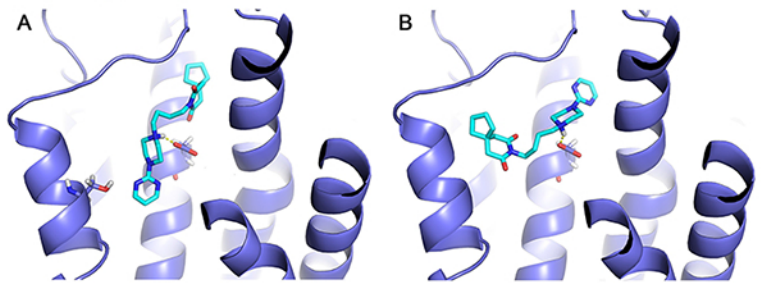


Table 1. Correlation between predicted and experimental binding data

	hD_3 (binding Energy Kcal/mol)		hD_2 (binding energy Kcal/mol)		Experimental K_i (nM) [pK]	
Ligand	AD4.2	DSX-Score	AD4.2	DSX-Score	hD_3	hD_2
Buspirone	-9.0	-122	-9.7	-97	8.04(Tadori et al. 2011) [8.1]	35.6(Tadori et al. 2011)[7.5]
SB277011A	-9.9	-127	-8.2	-66	11(Reavill et al. 2000) [7.9]	1032(Reavill et al. 2000) [6.0]
U99194A	-5.0	-104	-5.4	-87	160(Audinot et al. 1998)[6.8]	2281(Audinot et al. 1998) [5.6]

FIG 4. Binding of Buspirone at 5-HT_{1A} receptor model, predicted antagonist binding mode (A) and agonist (B) binding mode.



Conclusions:

This study demonstrates that D_3R is necessary for ethanol consumption in mice, because either D_3R gene deletion or D_3R pharmacological blockade by selective D_3R experimental antagonists or the approved drug buspirone, counteracted alcohol intake.

Molecular modeling has provided information about binding mode of different compounds with D_3 antagonist activity. Modeling and optimization of G protein receptor structures could be a valid approach helping in design and discovery of ligands with either selective or multi-pharmacological profile.

References:
[1] Rice OV, Patrick J, Schonhar CD, Ning H, Ashby CR, Jr. The effects of the preferential dopamine D(3) receptor antagonist S33138 on ethanol binge drinking in C57BL/6J mice. Synapse 2012; 66(11): 975-978.
[2] Platania CB, Salomone S, Leggio GM, Drago F, Bucolo C. Homology modeling of dopamine d(2) and d(3) receptors: molecular dynamics refinement and docking evaluation. PLoS One 2012; 7(9): e44316.
[3] Platania CB, Leggio GM, Drago F, Salomone S, Bucolo C. Regulation of intraocular pressure in mice: Structural analysis of dopaminergic and serotonergic systems in response to cabergoline. Biochem Pharmacol. 2013 Nov 1;86(9):1347-56.

DISCLOSURE:
The authors declare that there is no conflict of interest.
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