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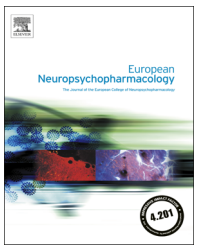

Dopamine D3 receptor-dependent changes in alpha6 GABAA subunit expression in striatum modulate anxiety-like behaviour: Responsiveness and tolerance to diazepam

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Abstract
Increasing evidence indicates that central dopamine (DA) neurotransmission is involved in pathophysiology of anxiety, in particular the DA receptor subtype 3 (D_3R). We previously reported that D_3R null mice ($D_3R^{-/-}$) exhibit low baseline anxiety levels and that acutely administrated diazepam is more effective in $D_3R^{-/-}$ than in wild type (WT) when tested in the elevated plus maze test (EPM). Here we tested the hypothesis that genetic deletion or pharmacological blockade of D_3R affect $GABA_A$ subunit expression, which in turn modulates anxiety-like behaviour as well as responsiveness and tolerance to diazepam. $D_3R^{-/-}$ mice exhibited tolerance to diazepam (0.5 mg/kg, i.p.), assessed by EPM, as fast as after 3 day-treatment, performing similarly to untreated $D_3R^{-/-}$ mice; conversely, WT exhibited tolerance to diazepam after a 14–21 day-treatment. Analysis of $GABA_A$ α6 subunit mRNA expression by qPCR in striatum showed that it was about 15-fold higher in $D_3R^{-/-}$ than in WT. Diazepam

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treatment did not modify α6 expression in $D_3R^{-/-}$, but progressively increased α6 expression in WT, to the level of untreated $D_3R^{-/-}$ after 14–21 day-treatment. BDNF mRNA expression in striatum was remarkably (>10-fold) increased after 3 days of diazepam-treatment in both WT and $D_3R^{-/-}$; such expression level, however, slowly declined below control levels, by 14–21 days. Following a 7 day-treatment with the selective D_3R antagonist SB277011A, WT exhibited a fast tolerance to diazepam accompanied by a robust increase in α6 subunit expression. In conclusion, genetic deletion or pharmacological blockade of D_3R accelerate the development of tolerance to repeated administrations of diazepam and increase α6 subunit expression, a $GABA_A$ subunit that has been linked to diazepam insensitivity. Modulation of $GABA_A$ receptor by DA transmission may be involved in the mechanisms of anxiety and, if occurring in humans, may have therapeutic relevance following repeated use of drugs targeting D_3R.

1. Introduction

Increasing evidence indicates that dopamine (DA) neurotransmission is involved in the pathophysiology of anxiety, in particular, a large literature points to a correlation between anxiety-like behavior and the mesolimbic DA pathway (Cabib and Puglisi-Allegra, 1994; Kienast et al., 2008; Talalaenko et al., 1994). DA exerts its action through five G protein-coupled receptor subtypes ($D_{1\text{-}5}R$); D_3R has an important role in the modulation of the mesolimbic DA pathway and in the control of several DA-related disorders such as addiction, depression and anxiety (Joyce and Millan, 2005; Leggio et al., 2014). The D_3R is, in fact, highly represented both at pre- and post-synapses, in the ventral striatum (nucleus accumbens and island of Calleja) (Bouthenet et al., 1991; Murray et al., 1994). In fact, D_3R is expressed also by tyrosine hydroxylase positive neurons, corresponding to its role as autoreceptor (Diaz et al., 2000). This is consistent with reports that mutant mice lacking D_3R receptors are hyperactive (Xu et al., 1997), presumably due to increases in DA resulting from a lack of negative feedback normally mediated through D_3R autoreceptors (Levant, 1997; Song et al., 2012b). Dopamine acting through the D_3R, may modulate the expression of innate anxiety-like behaviors involving a long-lasting, dynamic-dependent down-regulation of GABAergic control over lateral/basolateral amygdala neurons (Diaz et al., 2011). Such a D_3R-mediated, dynamic-dependent inhibition of $GABA_A$ receptor has also been found in the nucleus accumbens (NAc) (Chen et al., 2006) and hippocampus (Hammad and Wagner, 2006; Swant et al., 2008). However, the precise role of the D_3R/$GABA_A$ systems interaction both in the modulation of anxiety-like behaviors and the effect of anxiolytic drugs have not been completely explored. In a previous study, we found that $D_3R^{-/-}$ mice are more sensitive to the anxiolytic effect of diazepam than their WT littermates, which suggested potential alterations in the $GABA_A$ transmission.

In the present study, we tested the hypothesis that genetic deletion or pharmacological blockade of D_3R affect $GABA_A$ subunit expression, which in turn may modulate anxiety-like behaviour as well as responsiveness and tolerance to diazepam. In this respect, we assessed the behavioral response of $D_3R^{-/-}$ mice and their WT littermates, tested in the elevated plus maze (EPM), an experimental model of anxiety, and their sensitivity to repeated administration of diazepam. At the end of behavioral experiments, we analyzed the expression of $GABA_A$ receptor subunit α6 mRNA in the ventral striatum, a brain area where D_3R is predominantly expressed. Similarly, we assessed the behavioral response and the sensitivity to diazepam in WT mice following repeated treatment with SB277011A, a selective D_3R antagonist. Finally, since D_3R expression is related to brain-derived neurotrophic factor (BDNF) (Guillin et al., 2001; Le Foll et al., 2005; Leggio et al., 2014), and BDNF is involved in the control of $GABA_A$ receptor response in NAc (Koo et al., 2014), we also analyzed the expression of BDNF and D_3R.

2. Experimental procedures

2.1. Animals

Mice D_3R null ($D_3R^{-/-}$) and their WT littermates (males, 8–12 weeks old) were group-housed (3–5 mice per cage), with free access to chow and water, in an air-conditioned room, with a 12 h light–dark cycle. Mice $D_3R^{-/-}$ were 10th–12th generation of congenic C57BL/6 J mice, generated by a back crossing strategy (Accili et al., 1996). The genotypes of the $D_3R^{-/-}$ and WT mice were assessed by a PCR method with two pairs of primers flanking either exon 3 of the wild-type D_3R or the phosphoglycerate kinase 1 gene promoter cassette of the mutated gene (Accili et al., 1996).

All experiments were carried out according to the Directive 2010/63/EU and to the Institutional Animal Care and Use Committee of the Catania University.

2.2. Drugs and treatments

All drugs were purchased from Sigma (St Louis, MO). Diazepam was dissolved in physiological saline containing Tween 80 (0.1%), SB277011A hydrochloride was dissolved in physiological saline containing dimethyl sulfoxide (10%). All drugs were intraperitoneally (i.p.) injected (in a volume of 10 ml/kg). SB277011A was used at 10 mg/kg (Song et al., 2012a), diazepam was used at 0.5 mg/kg (Leggio et al., 2011).

All animals were gently manipulated by experienced facility keepers to avoid any environmental or physical stresses. In a first set of experiments $D_3R^{-/-}$ and WT were randomly allocated to the 10 experimental groups (n=6/8 per group): WT/naïve, $D_3R^{-/-}$/naïve, WT/vehicle single injection (SI), WT/diazepam SI, $D_3R^{-/-}$/vehicle SI, $D_3R^{-/-}$/diazepam SI, WT/vehicle (3 days), WT/diazepam (3 days), $D_3R^{-/-}$/vehicle (3 days), $D_3R^{-/-}$/diazepam (3 days). For the 3-day treatment, the animals were i.p. injected