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Lack of GSK3β activation and modulation of synaptic plasticity by dopamine in 5-HT1A-receptor KO mice

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ABSTRACT

Psychiatric disorders are associated with excitation-inhibition (E-I) balance impairment in the prefrontal cortex. However, how the E-I balance is regulated is poorly known. The E-I balance of neuronal networks is linked to the action of numerous neuromodulators such as dopamine and 5-HT. We investigated the role of D2-receptors in tuning the E-I balance in a mouse model of anxiety, the 5-HT1A-receptor KO mice. We focused on synaptic plasticity of excitation and inhibition on layer 5 pyramidal neurons. We show that D2-receptor activation decreases the excitation and favors HFS-induced LTD of excitatory synapses via the activation of GSK3β. This effect is absent in 5-HT1A-receptor KO mice. Our data show that the fine control of excitatory transmission by GSK3β requires recruitment of D2-receptors and depends on the presence of 5-HT1A-receptors. In psychiatric disorders in which the number of 5-HT1A-receptors decreased, therapies should reconsider how serotonin and dopamine receptors interact and control neuronal network activity.

1. Introduction

The prefrontal cortex (PFC) is a brain region highly associated with cognition and psychiatric disorders (Harrison and Weinberger, 2005; Lewis and González-Burgos, 2008). It receives serotonergic innervations from the medial and dorsal raphe nuclei (Mamounas et al., 1991; Steinbusch, 1981) and dopaminergic innervations from the tegmental ventral area also project to the PFC (Carr and Sesack, 2000). Serotonin (5-HT) and dopamine (DA) dysregulation have been involved in various neuropsychiatric disorders such as major depression, anxiety and schizophrenia (Meltzer and Massey, 2011; Meltzer et al., 2003). These observations have led to the development of new therapeutic strategies such as the use of pharmacological tools targeting metabotropic receptors, namely the serotonergic 1A receptor (5-HT_{1A}R) and the dopaminergic D2-like receptor (D_2R) (Newman-Tancredi, 2010). In these psychiatric disorders, a dysregulation of the balance between excitation (E) and inhibition (I) has been proposed (Yizhar et al., 2011).

In mammalian cerebral cortex, the relationship between synaptic excitation and inhibition affects many cortical functions such as feature selectivity and gain at the neuronal level (Haider and McCormick, 2009; Isaacson and Scanziani, 2011). It is striking that pyramidal neurons show a stable E-I ratio in time despite fluctuating cortical activity levels. This stability is mainly supported by the organization of neuronal networks, which permit a recruitment of interneurons via feedforward and/or feedback projections. When excitation increases, inhibition increases proportionally through an enhanced recruitment of inhibitory neurons; this phenomenon is referred to the E-I balance which has been extensively reported in various cortical structures (Wehr and Zador, 2003; Wilent and Contreras, 2005; Haider et al., 2006; Le Roux et al., 2006; Poo and Isaacson, 2009; Zhang et al., 2011; Xue et al., 2014). Another important aspect is the fine control of the E-I balance by G-protein coupled receptors. For example, we have previously shown that in the mouse PFC, the E-I balance determined in layer 5 pyramidal neurons (L5PyNs), is based on 20% excitation and 80% inhibition and that this set-point is finely modulated by the activity of 5-HT1ARs (Meunier et al., 2013). 5-HT1ARs have been shown to be located on L5PyNs and GABAergic interneurons (Santana et al., 2009). We have shown that stimulation of the 5-HT1ARs decreases AMPAR currents, modulates NMDAR currents and favors LTD (Meunier et al. 2013). Dopamine is known to modulate synaptic plasticity towards either LTP through D1Rs activation or LTD through D2R activation (Otani, 2003; Matsuda

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et al., 2006; Kolomiets et al., 2009; Sheynikhovich et al., 2011; Bai et al., 2014; Meunier et al., 2015; Otani et al., 2015) In the PFC, histological studies have shown a distribution of dopamine receptors on both pyramidal neurons and interneurons throughout layers 2–6 with a prominent proportion in deep cortical layers (Negyessy and Goldman-Rakic, 2005; Santana et al., 2009). D2 agonists are known to reduce pyramidal cell excitability, NMDAR and AMPAR responses and to activate fast-spiking GABAergic interneurons leading to a reduction of excitation as a consequence of the increased activity of inhibitory synapses on pyramidal neurons (Gulledge and Jaffe, 1998; Tseng and O'Donnell, 2004, 2007). These observations suggest that dopamine could be a potentially important modulator of the E-I balance.

One of the mechanisms of action related to the regulation of neuronal activity by dopamine or 5-HT involved GSK3 (glycogen synthase kinase 3) (Beaulieu et al., 2009). GSK3 is a multifunctional serine/threonine kinase, well known to regulate glycogen metabolism, which also plays an important role in regulating the activity of several receptors (Chen et al., 2009). For example, GSK3 is present in pyramidal neurons which suggest its important role in the modulation of synaptic efficacy (Yoshimura et al., 2005). Two forms of GSK3 (GSK3α and GSK3β) have been identified (Woodgett, 1990, 2001). These kinases are constitutively active and can be inactivated through their phosphorylation by Akt on a single serine residue: serine 21 (GSK3α) or serine 9 (GSK3β) at the N-terminus (Cross et al., 1995; Frame et al., 2001). Moreover, it has been reported that the stimulation of D2Rs in the PFC and the striatum, induces an activation of GSK3 (Beaulieu and Gainetdinov, 2011; Beaulieu et al., 2004, 2008, Chen et al., 2009). This activation of GSK3β has been shown to promote the internalization of the NR2B subunit of NMDARs leading in the rat hippocampus to the induction of LTD (Peineau et al., 2007) while LTP could be favored by higher levels of phosphorylation of GSK3β (Hooper et al., 2007; Peineau et al., 2007). In the case of 5-HT, it has been reported that GSK3 regulation modulates NMDARs and AMPARs through either 5-HT1AR or 5-HT2AR activation (Li et al., 2004, 2007).

From these studies, it clearly appears that 5-HT1AR and D2R stimulation regulates GSK3 activity in a physiological situation. Importantly, similar to 5-HT1ARs and D2Rs, GSK3 is also a molecular target in the treatment of neuropsychiatric disorders such as major depression and anxiety (Meltzer and Massey, 2011; Meltzer et al., 2003). In our study, we used 5-HT1AR-KO mice described as a model for anxiety (Ramboz et al., 1998) that have been designed to better understand human psychiatric conditions where a profound decrease of 5-HT1AR expression has been demonstrated (Sargent et al., 2000; Bhagwagar et al., 2004; Shively et al., 2006; Lanzenberger et al., 2007; Akimova et al., 2009). Knowing the importance of dopamine in psychiatric treatment, we looked at whether the D2R effects on the neuronal activity and GSK3 signaling remains functional in 5-HT1AR-KO mice. We investigated the role of D2Rs and 5-HT1ARs in the prefrontal cortex to tune the E-I balance, to further understand the remodeling of the E/I balance that could occur in various psychiatric disorders (Yizhar et al., 2011). We also focused our work on synaptic plasticity of excitatory and inhibitory synapses on layer 5 pyramidal neurons. We show that D2R activation decreases the E-I balance and favors HFS-induced LTD of excitatory synapses via the activation of GSK3 and that D2R activation failed to modulate plasticity in 5-HT1AR-KO mice. From our data it appears that GSK3 modulation is impaired in 5-HT1AR-KO mice and suggests that GSK3 is central and required for a dual activation of D2Rs and 5-HT1ARs to control plasticity.

2. Methods

2.1. Animals

Paired wild-type 129/Sv and 5-HT1AR-KO mice (Ramboz et al., 1998) were bred under standard conditions in our animal facilities. All efforts were made to reduce the number of animals used. The agreement number for our animal facilities is: C 91-471-104. Our study includes exclusively *in vitro* experiments with no *in vivo* work. The experiments were done in accordance with the European and Institutional guidelines for the care and use of laboratory animals (Council Directive 86/609/EEC and 2010/63/UE) and its application in 2013 by the French National Research Council. The article 3 of the 2010/63/UE directive permits euthanasia of animals by cervical dislocation to excise brain tissues for experiments without any requirement of a specific ethical committee agreement. P21 to P28 mice were killed by cervical dislocation and their brains quickly removed.

Genotyping was carried out by polymerase chain reaction applied to genomic DNA from tail biopsies with appropriate primers. Wild-type (n = 38 animals) and mutant (n = 38 animals) mice were the product of mating between heterozygous couples raised on the same 129/Sv genetic background (Boutrel et al., 2002).

2.2. Protein preparation and immunoblotting

After decapitation, the PFC was rapidly dissected and homogenized in lysis buffer containing 10 mM Tris HCl pH 7.4, 150 mM NaCl, 1 mM EDTA, 1 mM EGTA, 0.5% NP-40, 1 μg/ml CLAP, 1mMPefabloc, 10 μg/ml aprotinin, 1 mM sodium vanadate, 50 ng/μl okadaic acid. The lysates were centrifuged at 13000*g* at 4 °C to remove insoluble debris. Proteins were separated by SDS-PAGE using an 8% polyacrylamide gel and transferred onto nitrocellulose membranes. Membrane were blocked with 2.5% skimmed milk and then incubated with primary antibodies to phospho-GSK-3a (Ser21), phospho-GSK-3b (Ser9) (Cell Signaling Technology) or *p*-GSK-3a/b, b-actin (AC-15) (Santa Cruz Biotechnology). Appropriate horseradish peroxidase-conjugated anti-mouse or anti-rabbit IgG as well as fluorescent Alexa Fluor 680-conjugated anti-mouse (A 21057, Invitrogen/Molecular Probes) or IRDye 800CW-conjugated anti-rabbit (926–32211,LI-COR Biosciences) IgG were used for detection. We used the two-color infrared imaging system from LI-COR Biosciences for quantification. The infrared fluorescent signals at 680 and 800 nm were recorded and quantified with an Odyssey scanner from LI-COR Biosciences.

The GSK3 β data have been normalized to values yielded with actin and expressed as percentage changes after treatments versus controls.

2.3. Slice preparation and electrophysiological recordings

Coronal slices (250 μm thickness) containing PFC were obtained from P21 to P28 mice. They were incubated for at least 1 h at 33 °C in the extracellular solution (ES) containing (in mM): NaCl, 126; $NaHCO_3$, 26; glucose, 10; $CaCl_2$, 2; KCl, 1.5; KH_2PO_4, 1.25; $MgCl_2$, 2 (pH 7.4, 310–330 mOsm). The ES was infused continuously with a mixture of 95% O_2 and 5% CO_2. Somatic whole-cell recordings of L5PyNs were performed at 33 °C, using borosilicate glass pipettes (of 3–5 MΩ resistance in bath) filled with a solution containing (in mM): K-gluconate, 140; HEPES, 10; ATP, 4; $MgCl_2$, 2; GTP, 0.4; EGTA, 0.5 (pH 7.3 adjusted with KOH; 270–290 mOsm). Voltage-clamp recordings were performed using a Multiclamp 700A (Axon Instruments), filtered by a low-pass Bessel filter with a cutoff frequency set at 2 kHz, and digitally sampled at 4 kHz. The membrane potential was corrected off-line by − 10 mV to account for the