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Sex-related neurogenesis decrease in hippocampal dentate gyrus with depressive-like behaviors in sigma-1 receptor knockout mice

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Abstract
Male sigma-1 receptor knockout ($\sigma_1R^{-/-}$) mice showed depressive-like phenotype with deficit in the survival of newly generated neuronal cells in the hippocampal dentate gyrus (DG), but female $\sigma_1R^{-/-}$ mice did not. The level of serum estradiol (E2) at proestrus or diestrus did not differ between female $\sigma_1R^{-/-}$ mice and wild-type (WT) mice. Ovariectomized (OVX) female $\sigma_1R^{-/-}$ mice, but not WT mice, presented the same depressive-like behaviors and neurogenesis decrease as male $\sigma_1R^{-/-}$ mice. Treatment of male $\sigma_1R^{-/-}$ mice with E2 could alleviate the depressive-like behaviors and rescue the neurogenesis decrease. In addition, E2 could correct the decline in the density of NMDA-activated current (I_{NMDA}) in granular cells of DG and the phosphorylation of NMDA receptor (NMDAr) subtype 2B (NR2B) in male $\sigma_1R^{-/-}$ mice, which was associated with the elevation of Src phosphorylation. The neuroprotection and antidepressant effects of E2 in male $\sigma_1R^{-/-}$ mice were blocked by the inhibitor of Src or NR2B. The NMDAr agonist showed also the neuroprotection and antidepressant effects in male $\sigma_1R^{-/-}$ mice, which were insensitive to the Src inhibitor. On the other hand, either the deprivation of E2 or the inhibition of Src in female $\sigma_1R^{-/-}$ mice rather than WT mice led to a distinct decline in I_{NMDA} and NR2B phosphorylation. Similarly, the Src inhibitor could cause neurogenesis decrease and depressive-like behaviors in female $\sigma_1R^{-/-}$ mice, but not in WT mice. These results indicate that the σ_1R deficiency impairs neurogenesis leading to a depressive-like phenotype, which is alleviated by the neuroprotection of E2.

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1. Introduction

Sigma-1 receptors (σ_1R) are highly expressed in regions of brain involved in emotion and neuropsychiatric disorders (Maurice et al., 2002). The σ_1R agonists are a known class of drugs for the treatment of depression (Urani et al., 2001) and anxiety (Longone et al., 2011). The σ_1R knockout ($\sigma_1R^{-/-}$) mice exhibit a depressive-like phenotype (Sabino et al., 2009). However, the underlying mechanisms have not yet been fully elucidated.

Growing evidence suggests that neurogenesis continues throughout adulthood within the hippocampal dentate gyrus (DG). Adult-generated neurons can integrate into the hippocampal circuitry to maintain functional structure (Toni et al., 2008), which is required for mood control and antidepressant efficacy (Petrik et al., 2012). Clinical trials have proven that the hippocampal volume in patients with depression is smaller than that in normal subjects of the same age (Videbech and Ravnkilde, 2004). Electroconvulsive therapy in refractory depression can increase the hippocampal volume (Tendolkar et al., 2013). The activation of the *N*-methyl-D-aspartate receptor (NMDAr) is important for the survival and/or circuit integration of newborn neurons in the hippocampal DG (Tashiro et al., 2006). The activation of σ_1R potentiates the NMDAr currents by preventing small conductance Ca^{2+}-activated K^+ channels (Martina et al., 2007) and enhancing NMDAr trafficking to the plasma membrane (Pabba et al., 2014). The activation of σ_1R increases the Ca^{2+} influx across the NMDAr through enhancing phosphorylation of the NMDAr NR2B subunit (Cai et al., 2008; Chen et al., 2007). Selective σ_1R ligands can increase NMDA-induced [^{3}H] norepinephrine release and neuronal firing (Monnet and Maurice, 2006) in a protein kinase C-dependent manner (Nuwayhid and Werling, 2003). An earlier study (Yang et al., 2011) reported that σ_1R agonist enhances the survival of newborn neurons in an NMDAr-dependent manner. The σ_1R deficiency suppresses the NR2B phosphorylation leading to the dysfunction of NMDAr without the changes in membrane properties, which impairs the survival of newborn neurons in the DG (Sha et al., 2013). The density of NMDAr is lower in the brain of depressed patients compared to controls (Nudmamud-Thanoi and Reynolds, 2004; Nowak et al., 1995). Thus, the affective disorders in $\sigma_1R^{-/-}$ mice are related to the decline of neurogenic capacity in the hippocampal DG.

Interestingly, the depressive-like phenotype is observed in male $\sigma_1R^{-/-}$ mice but not in females (Chevallier et al., 2011). A reduction of mature newborn neurons has been determined in male $\sigma_1R^{-/-}$ mice (Sha et al., 2013). During pregnancy, the number of newborn neurons in the DG is significantly increased followed by a rapid decline after parturition (Pawluski and Galea, 2007; Furuta and Bridges, 2005). The change in the number of newly generated neurons in the DG has been related to the fluctuation in serum estrogen levels (Tanapat et al., 1999). Activation of the estrogen receptor (ER) can enhance the hippocampal neurogenesis (Brannvall et al., 2002). In male meadow voles, treatment with estradiol (E2) is able to enhance the survival of young neurons (Ormerod et al., 2004). Replacement therapy of E2 is beneficial to women who suffer from postpartum and perimenopausal affective disorders (Cohen et al., 2003). Thus, it is of great interest to investigate the hippocampal neurogenesis in female $\sigma_1R^{-/-}$ mice.

The present study employed adult male and female $\sigma_1R^{-/-}$ mice and focused on exploring the correlation between neurogenesis decrease and depressive-like phenotype. First, the process of neurogenesis was examined in the hippocampal DG of male and female $\sigma_1R^{-/-}$ mice. After assessing stress hormones and sex hormones, we further investigated the involvement of E2 in the neurogenesis decrease and depressive-like behaviors of $\sigma_1R^{-/-}$ mice and the underlying mechanisms. Finally, we tested whether the neurogenesis decrease in $\sigma_1R^{-/-}$ mice was related to their depressive-like phenotype.

2. Experimental procedures

2.1. Animals

All animal experiments were approved by the Institutional Animal Care and Ethical Committee of Nanjing Medical University and were performed in accordance with experimental animal guidelines of the Laboratory Animal Research Institute. All efforts were made to minimize animal suffering and to reduce the number of animals used. The σ_1R knockout ($\sigma_1R^{-/-}$) mice were generated and characterized as described previously (Sabino et al., 2009). Eight-week-old male and female $\sigma_1R^{-/-}$ mice and wild-type (WT) littermates were used at the beginning of the experiment. Animals were housed in plastic cages at 23 ± 2 °C and 55% relative humidity with a 12:12 h light/dark cycle in the Animal Research Center of Nanjing University. Water and food were given ad libitum.

2.2. Administration of drugs

The NR2B inhibitor Ro25-6981, Src inhibitor PP2 and NMDAr agonist NMDA were purchased from Sigma-Aldrich (St. Louis, MO, USA). The Src inhibitor dasatinib was obtained from Selleck Chemicals (Munich, Germany). Ro25-6981 and NMDA were dissolved in 0.9% sterile saline. PP2 and dasatinib were dissolved in dimethyl sulfoxide (DMSO) and diluted in sterile saline to a final concentration of 1.0% DMSO. Ro25-6981 (6 mg/kg; Yin et al., 2015), NMDA (30 mg/kg; Sha et al., 2013), PP2 (0.03 mg/kg; Schumann et al., 2008) or dasatinib (0.5 μg/kg; Dhawan and Combs, 2012) was intraperitoneally (i.p.) injected daily. Male mice were implanted with a subcutaneous pellet containing estradiol (E2, 0.025 mg; Innovative Research of America, Sarasota FL) at the nape of the neck to maintain a physiologic level of serum E2 (Horsburgh et al., 2002). Vehicle at the same volume was administered as a control.

2.3. Ovariectomy (OVX) treatment

Bilateral ovaries were removed in female mice under anesthesia (chloral hydrate, 400 mg/kg, i.p.). Briefly, the dorsal surface was shaved and the vascular supply was ligated. Then, the ovaries were dissected out through bilateral incisions. Sham-operated control mice were subjected to surgery in which the ovaries were manipulated but left intact.

2.4. Behavioral examination

Tail suspension test (TST): Mice were moved to the testing area in their home cages and allowed to adapt to the new environment for at least 1 h. Mice were suspended by the tail using adhesive tape to a rod 60 cm above the floor. Trials were conducted for a period of 6 min and were video recorded (Zhou et al., 2011). The behavioral

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