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Research report

Role of proBDNF and BDNF in dendritic spine plasticity and depressive-like behaviors induced by an animal model of depression

Hui Qiao [a], Shu-Cheng An [a,*], Chang Xu [a], Xin-Ming Ma [a,b]

[a] College of Life Science, Shaanxi Normal University, Xi'an, Shaanxi Province 710062, PR China
[b] University of Connecticut Health Center, Department of Neuroscience, Farmington, CT 06030, USA

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ABSTRACT

Major depressive disorder (MDD) is one of the most common psychiatric disorder, but the underlying mechanisms are largely unknown. Increasing evidence shows that brain-derived neurotrophic factor (BDNF) plays an important role in the structural plasticity induced by depression. Considering the opposite effects of BDNF and its precursor proBDNF on neural plasticity, we hypothesized that the balance of BDNF and proBDNF plays a critical role in chronic unpredicted mild stress (CUMS)-induced depressive-like behaviors and structural plasticity in the rodent hippocampus. The aims of this study were to compare the functions of BDNF and proBDNF in the CUMS-induced depressive-like behaviors, and determine the effects of BDNF and proBDNF on expressions of kalirin-7, postsynaptic density protein 95 (PSD95) and NMDA receptor subunit NR2B in the hippocampus of stressed and naïve control rats, respectively. Our results showed that CUMS induced depressive-like behaviors, caused a decrease in the ratio of BDNF/proBDNF in the hippocampus and resulted in a reduction in spine density in hippocampal CA1 pyramidal neurons; these alterations were accompanied by a decrease in the levels of kalirin-7, PSD95 and NR2B in the hippocampus. Injection of exogenous BDNF into the CA1 area of stressed rats reversed CUMS-induced depressive-like behaviors and prevented CUMS-induced spine loss and decrease in kalirin-7, NR2B and PSD95 levels. In contrast, injection of exogenous proBDNF into the CA1 region of naïve rats caused depressive-like behavior and an accompanying decrease in both spine density and the levels of kalirin-7, NR2B and PSD95. Taken together, our results suggest that the ratio of BDNF to proBDNF in the hippocampus plays a key role in CUMS-induced depressive-like behaviors and alterations of dendritic spines in hippocampal CA1 pyramidal neurons. Kalirin-7 may play an important role during this process.

1. Introduction

Depression is a severe psychiatric disorder that affects up to 7% of the population in the United States (Levinstein and Samuels, 2014). Although depression has been studied for several decades, the underlying mechanisms which cause depression are still not clear. Some depression patients are resistant to currently available antidepressant treatments. In order to develop more effective treatments, it is important to identify the mechanisms underlying depression. Accumulating evidence shows that structural plasticity the neurons, particularly atrophy of dendrites and loss of dendritic spines in hippocampal neurons, may play a key role in the pathogenesis of depression (von Bohlen Und Halbach, 2009; Mandela and Ma, 2012; Duman and Duman, 2015).

It is well documented that depression-mediated structural plasticity in the hippocampus is associated with alterations in brain-derived neurotrophic factor (BDNF) signaling (Duman and Monteggia, 2006; Castren and Rantamaki, 2010), however the role of its precursor (proBDNF) has not been clearly elucidated. BDNF is initially synthesized as a precursor protein (proBDNF) (Matsumoto et al., 2008). BDNF has been shown to promote neuronal survival and differentiation and increase formation of dendritic spines and synapses (Tyler and Pozzo-Miller, 2003; Hiester et al., 2013) via its high-affinity receptor, tropomyosin-related kinase B (TrkB) (Huang and Reichardt, 2003). However, ProBDNF causes a simplification of the dendritic arbor, decreases spine density (Zagrebelsky et al., 2005; Yang et al., 2014), and induces long-term depression (LTD) (Woo et al., 2005) through its p75 neurotrophin receptor (p75NTR) in hippocampal neurons.

Abbreviations: BDNF, brain-derived neurotrophic factor; MDD, major depressive disorder; CUMS, chronic unpredicted mild stress; NR2B, NMDA receptor subtype 2B; PSD95, postsynaptic density protein 95; TrkB, tropomyosin-related kinase B.

* Corresponding author at: Mailbox 100, 620 West Chang'an Street, Shaanxi Normal University, Xi'an, Shaanxi Province 710119, PR China.
E-mail address: shuchengan@snnu.edu.cn (S.-C. An).

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Chronic unpredictable mild stress (CUMS) has been used as an established animal model of depression (Qiao et al., 2016). The mechanisms through which CUMS causes structural and functional alterations in the hippocampus are not clear. Considering the opposite functions of BDNF and proBDNF in neuronal plasticity (Martinowich et al., 2007; Je et al., 2012) and the conflicting reports about CUMS's ability to reduce BDNF levels in the hippocampus (Allaman et al., 2008; Larsen et al., 2010), we hypothesized that the balance of proBDNF and BDNF in the hippocampus plays a key role in the development of CUMS-induced depressive-like behaviors.

Kalirin-7, the most abundant kalirin isoform in the adult rodent brain, is exclusively localized to the postsynaptic side of excitatory synapses (Ma et al., 2008a,b; Ma et al., 2011). Kalirin-7 which interacts with many synaptic proteins including PSD95 and NR2B in the postsynaptic side of excitatory synapses plays a key role in spine morphogenesis and synaptic plasticity (Penzes et al., 2001; Ma et al., 2008a,b; Kiraly et al., 2011; Mandela and Ma, 2012). Hippocampal CA1 pyramidal neurons of kalirin-7 knockout (KO) mice show a decrease in spine density and deficit in long-term potentiation (LTP). Accumulating evidence demonstrates that chronic stress results in a decrease in spine density in the pyramidal neurons of the hippocampal CA3 (Magarinos et al., 1996; Sousa et al., 2000; Sandi et al., 2003; Pawlak et al., 2005; Chen et al., 2010; Qiao et al., 2014) or CA1 (Kassem et al., 2013; Qiao et al., 2014; Castaneda et al., 2015; Huang et al., 2015) or both CA1 and CA3 (Hajszan et al., 2009; Magarinos et al., 2011; Kim et al., 2013) regions. Our previous study showed that a CUMS-mediated decrease in spine density in CA1 and CA3 pyramidal neurons is accompanied by a decrease in the levels of kalirin-7 (Qiao et al., 2014). Kalirin has been identified as a downstream target of BDNF in cultured neurons *in vitro* (Yan et al., 2016). Although the roles of BDNF and kalirin-7 in spine formation are well established (Tyler and Pozzo-Miller, 2003; Mandela and Ma, 2012; Ma et al., 2014), it remained unknown how BDNF or proBDNF regulates kalirin-7 expression in the hippocampus after exposure to CUMS. Therefore, we hypothesized that the ratio of BDNF/proBDNF plays an important role in CUMS-induced depressive-like behaviors and CUMS-mediated decreases in spine density in hippocampal neurons. Kalirin-7 may work as a downstream signal of BDNF and proBDNF to regulate CUMS-mediated spine plasticity.

To test our hypothesis, we determined the role of the BDNF/proBDNF ratio in CUMS-induced depressive-like behaviors and spine loss and examined the expression of kalirin-7 and its binding partners, including PSD95 and NR2B, in the hippocampus after exposure to CUMS or pretreatment with proBDNF in rat. The experimental design is showed in Fig. 1.

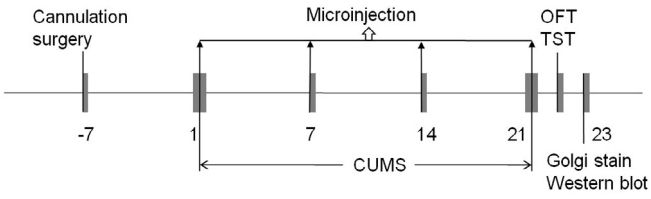

Fig. 1. Experimental design. CUMS: 21 days of chronic unpredicted mild stress (CUMS). OFT: open field test on day 22. TST: tail suspension test on day 22. Microinjection: CUMS animals received microinjections of saline (the CUMS group) or BDNF (the CUMS + BDNF group) into the CA1 region on day 1, 7, 14 and 21 after the onset of CUMS, respectively; naïve rats in control group received microinjections of saline (control group) or proBDNF (the proBDNF group) into the CA1 region on day 1, 7, 14 and 21, respectively. Rats received the cannulation surgery 7 days before the onset of CUMS.

2. Results

2.1. CUMS-induced depressive-like behaviors in rats

The open field test (OFT) is one of the most widely used tests for estimating the Anxiety related behaviors of rodents, and is commonly used for measuring general locomotor activity, exploratory behavior (rearing) and self-care behavior (grooming) in rodents (Luo et al., 2008). As expected, CUMS decreased the locomotor activity (116.1 ± 17.7 in the CUMS group *vs.* 144.5 ± 16.5 of control, $n = 8$, $p < 0.05$, Fig. 2A), the number of rearings (6.7 ± 1.5 in the CUMS group *vs.* 14.5 ± 2.4 in the control group, $n = 8$. $p < 0.01$, Fig. 2B) and grooming behavior (4.0 ± 1.2 in the CUMS group *vs.* 9.5 ± 1.7 of control, $n = 8$, $p < 0.01$, Fig. 2C) in the open field test. The tail suspension test (TST) is used to assess moods in rodents (Guan et al., 2015). A longer immobility time indicating a depressive-like behavior. In agreement with our previous report (Qiao et al., 2014), the tail suspension test showed that CUMS increased the duration of immobility (135.2 ± 13.2 s in the CUMS group *vs.* 70.6 ± 7.1 s in the control group, $n = 8$, $p < 0.01$, Fig. 2D).

2.2. CUMS decreased the ratio of BDNF/proBDNF by decreasing BDNF expression without altering proBDNF level in the hippocampus

Western blot analysis showed that the expression of BDNF in the hippocampus decreased in the CUMS group compared with the control group (83.3 ± 4.6% of control, $n = 8$, $p < 0.05$, Fig. 2). Though the expression of proBDNF tended to increase in the CUMS group, it did not reach a statistically significant difference compared with the control group (109.2 ± 9.9% of control, $n = 8$, $p > 0.05$, Fig. 3). The ratio of BDNF/proBDNF was significantly decreased in the CUMS group (70.1 ± 4.7% of control, $n = 8$, $p < 0.01$, Fig. 2B).

2.3. CUMS-induced depressive-like behaviors were partially attenuated by microinjection of BDNF into hippocampal CA1 region, while microinjection of proBDNF into the same area induced depressive-like behaviors in naïve control rats

Microinjection of BDNF into the rat CA1 area in the CUMS group reversed CUMS-induced decreases in locomotor activity (140.2 ± 10.4 in the CUMS + BDNF group *vs.* 116.1 ± 17.7 in the CUMS group, $n = 8$, $p < 0.05$; Fig. 2A), the numbers of rearings (9.0 ± 1.6 in the CUMS + BDNF group *vs.* 6.7 ± 1.5 in the CUMS group, $n = 8$, $p < 0.05$, Fig. 2B), and the duration of immobility (66.7 ± 10.1 s in the CUMS + BDNF group *vs.* 135.3 ± 13.3 s in the CUMS group, $n = 8$, $p < 0.01$, Fig. 2D) compared with the CUMS group which received saline only. Interestingly, microinjection of proBDNF into the CA1 region of naïve control rats induced depressive-like behaviors characterized by lower locomotor activity (108.1 ± 14.0 in the proBDNF group *vs.* 144.5 ± 16.5 in the control group, $n = 8$, $p < 0.01$, Fig. 2A), decreased rearing activity (5.2 ± 1.6 in the proBDNF group *vs.* 14.5 ± 2.4 in the control group, $n = 8$, $p < 0.01$, Fig. 2B) and decreased grooming behavior (2.1 ± 1.0 in the proBDNF group *vs.* 9.5 ± 1.7 in the control group, $n = 8$, $p < 0.01$, Fig. 2C) as well as an increased time of immobility in the proBDNF group compared with the control group (138.5 ± 6.7 in the proBDNF group *vs.* 70.6 ± 7.1sec in the control group, $n = 8$, $p < 0.01$) (Fig. 2D).

2.4. CUMS-induced decrease in spine density in CA1 pyramidal neurons was reversed by injection of BDNF into CA1 area while injection of proBDNF into the same area decreased spine density in CA1 pyramidal neurons in native control rats

To compare the role of BDNF and proBDNF on dendritic spines, we counted the dendritic spine density of hippocampal CA1