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Epigenetic regulation of BDNF in the learned helplessness-induced animal model of depression

Chun-Lin Su [1], Chun-Wei Su [1], Ya-Hsin Hsiao, Po-Wu Gean*

Department of Pharmacology, College of Medicine, National Cheng-Kung University, Tainan 701, Taiwan

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ABSTRACT

Major depressive disorder (MDD), one of the most common mental disorders, is a significant risk factor for suicide and causes a low quality of life for many people. However, the causes and underlying mechanism of depression remain elusive. In the current work, we investigated epigenetic regulation of BDNF in the learned helplessness-induced animal model of depression. Mice were exposed to inescapable stress and divided into learned helplessness (LH) and resilient (LH-R) groups depending on the number they failed to escape. We found that the LH group had longer immobility duration in the forced swimming test (FST) and tail suspension tests (TST), which is consistent with a depression-related phenotype. Western blotting analysis and enzyme-linked immunosorbent assay (ELISA) revealed that the LH group had lower BDNF expression than that of the LH-R group. The LH group consistently had lower *BDNF* mRNA levels, as detected by qPCR assay. In addition, we found *BDNF* exon IV was down-regulated in the LH group. Intraperitoneal injection of imipramine or histone deacetylase inhibitors (HDACi) to the LH mice for 14 consecutive days ameliorated depression-like behaviors and reversed the decrease in BDNF. The expression of HDAC5 was up-regulated in the LH mice, and a ChIP assay revealed that the level of HDAC5 binding to the promoter region of *BDNF* exon IV was higher than that seen in other groups. Knockdown of HDAC5 reduced depression-like behaviors in the LH mice. Taken together, these results suggest that epigenetic regulation of BDNF by HDAC5 plays an important role in the learned helplessness model of depression.

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1. Introduction

Most people recover from negative emotions sooner or later, whereas depressive patients usually maintain a low mood for a long period of time. Major depressive disorder (MDD) is one of the most common mental disorders around the world, and one that causes patients to feel emotional suffering and endure a low quality of life. Learned helplessness (LH), an animal model of depression, describes a paradigm in which animals stop trying to escape an aversive situation after repeatedly experiencing inability to escape. Interestingly, however, although a group of the same animals may suffer from the same aversive conditioning, some animals will have the opposite responses, and still continue to try to escape. However, the behavior of stopping to try in an aversive situation is similar to the performance of depressive patients (Overmier and Seligman, 1967; Chourbaji et al., 2005; Abelaira et al., 2013).

Brain-derived neurotrophic factor (BDNF) plays a crucial role in brain development, and also is associated with neuroplasticity (Korte et al., 1995; Pang et al., 2004) and neuroprotection (Hetman et al., 1999). In multiple stress-induced animal models of depression, it has been shown that BDNF is down-regulated in the hippocampus of animals with depression-like phenotype (Nibuya et al., 1995; Smith et al., 1995; Pizarro, 2004). Clinical studies have also revealed that depressive patients have lower concentrations of serum BDNF (Bocchio-Chiavetto et al., 2010; Yoshida et al., 2012). In parallel, local infusions of BDNF to the hippocampus can have an antidepressant-like effect (Nibuya et al., 1995; Shirayama et al., 2002). However, selective knockout of BDNF in the hippocampus by combining the adeno-associated virus (AAV) with the Cre/loxP site-specific recombination system did not alter depression-related behaviors (Adachi et al., 2008; Monteggia et al., 2007; but see Taliaz et al., 2010, 2013). Moreover, although BDNF is down-regulated in the hippocampus in many multiple stress-induced animal models of depression (Nibuya et al., 1995; Smith

* Corresponding author.
E-mail address: powu@mail.ncku.edu.tw (P.-W. Gean).
[1] These authors contributed equally to this work.

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et al., 1995; Pizarro, 2004), some studies reported an increase while others reported no substantial changes in BDNF expression following chronic social defeat (Coppens et al., 2011; Taliaz et al., 2011, 2013). These discrepancies could be attributed to differences in experimental designs, the animal models used or the hippocampal subregions analyzed.

Epigenetic mechanisms, which can regulate gene expression without altering DNA sequences, provide platforms for investigating gene–environment interactions. A growing body of evidence suggests that epigenetic dysregulation may contribute to neuropsychiatric disorders (Tsankova et al., 2007; Nestler, 2014). In mice, chronic defeat induced down-regulation of *BDNF* exons III and IV, likely through hyper-methylation of H3K27 around their respective exons (Tsankova et al., 2006). Chronic antidepressant treatment reversed this down-regulation, increased histone acetylation and decreased expression of histone deacetylase 5 (HDAC 5). Furthermore, overexpression of HDAC5 in the hippocampus blocked the antidepressant effect of imipramine. Consistently, by promoting acetylation of histones H3 and H4 of *BDNF* exons 1 and 4, HDAC inhibitors exhibited an antidepressant-like effect (Koppel and Timmusk, 2013) similar to that of imipramine (Tsankova et al., 2006).

HDAC proteins are classified into four classes based on function and DNA sequence homology (Kurdistani and Grunstein, 2003). Classes I, II and IV have a zinc dependent active site, whereas Class III contains family of NAD^+-dependent deacetylase known as sirtuins (de Ruijter et al., 2003; Barneda-Zahonero and Parra, 2012). The results of a mapping study showed that HDACs 2, 3, 4, 5 and 11 were highly expressed in adult rat brains (Broide et al., 2007). The purpose of this study was to investigate factor(s) which could account for resilience to learned helplessness-induced depression-like behavior. We found that protein and mRNA levels of hippocampal BDNF were significantly lower in the LH group, whereas the level of HDAC5 binding to the promoter of *BDNF* exon IV was significantly higher. Knockdown of HDAC5 reduced depressive behavior in the LH mice, suggesting that epigenetic regulation of BDNF by HDAC5 plays an important role in the learned helplessness model of depression.

2. Materials and methods

2.1. Animals

Male C57BL/6 strain mice weighing 22–25 g (8 weeks) were purchased from the National Laboratory Animal Centre (NLAC) in Taiwan. Mice were group-housed in Plexiglas cages (27.5 cm × 15.5 cm × 18.5 cm) with a humidity of 55% ± 5%, room temperature of 21 °C ± 2 °C and 12 h light/dark cycle. The animals were allowed access to food and water *ad libitum*. All tests were carried out blinded and the animal codes were shown only at the end of the analysis. All animal care, experiments and procedures were approved by the Institutional Animal Care and Use Committee (IACUC) of National Cheng Kung University.

2.2. Drug administration

All drugs were purchased from Sigma (Sigma, St. Louis, MO, USA). For antidepressant treatment, imipramine at the doses of 5, 10 and 15 mg/kg was intraperitoneally injected daily twice to the mice, lasting from day 6 to day 19, for 14 consecutive days (Mlyniec and Nowak, 2013; Nowak et al., 1991; Song et al., 2006). SB or VPA at dose of 600 mg/kg was intraperitoneally injected to the mice once daily, running from day 6 to day 19 (Han et al., 2014; Ookubo et al., 2013; Schroeder et al., 2007).

2.3. The learned helplessness animal model

The LH paradigm was modified from a previous report (Song et al., 2006). On the first day, each mouse was placed in the two-way shuttle box (26 × 23 × 16 cm) for 2 min in order to habituate them to the environment, and the mouse was subsequently given five footshocks in five trials (0.6 mA; 2 s in duration; interval of 30 s). On day 1 of the escape screening test, the mice could escape to the other side of the box when they received footshocks. All the mice were then divided into experimental and control groups. On days 2–4, the mice in experimental group were subjected to a training protocol with 60 inescapable footshocks (0.6 mA; 15 s in duration; mean interval of 45 s). In contrast, the control group stayed in the same environment on days 2–4 without receiving footshocks. On day, each mouse was placed in a shuttle box that was identical to the one used on day 1 and exposed to 30 footshocks (0.6 mA; 2 s in duration; interval of 30 s). The definition of a failed escape was that the mice did not move to the other side of the box in the 30 s after the each footshock. All behaviors were recorded by camera, and the video were analyzed by an observer to calculate the number of times each mouse failed to escape. The mice received footshocks during days 2–4 and performed escaped failure times more than 25 on day 5 were regarded as learned helplessness (LH) group, while the others were classified as learned helplessness resilient (LH-R) group.

2.4. Forced swimming test (FST)

The FST was conducted as in previous studies, albeit with some modifications (Adachi et al., 2008; Berry et al., 2012). Mice were individually forced to swim for 10 min for two consecutive days in an inescapable vertical translucent acrylic cylinder (25 cm tall × 19 cm diameter) containing 20 cm of clear water (19–21 °C). The entire process was videotaped using a Sony digital camera (HDR-XR 150). The total duration of immobility, as an index of depressive behavior, was timed only on the last day by a trained observer. Immobility was defined as the animal making no struggle except those motions necessary to hold their head above water. Mice groups were blinded to the observer until the end of analysis.

2.5. Tail suspension test (TST)

The mice were individually suspended by the tail above the ground, with adhesive tape placed about 1–1.5 cm from the tip. The courses of the TST over a single 6-min session were recorded with a Sony digital camera (HDR-XR 150). The total time spent immobile during the last 4 min of a session were scored by a trained observer blinded to the group assignments. Immobility was defined as the mice only hanging passively without any active movements, and remaining entirely motionless. The body may be curled upward or straight down depending on the individual. Some of the mice performed swinging behavior, and this was considered as an active state, rather than immobility. Tests were conducted between 9:00 and 18:00. Previous studies showed that some of C57BL/6 mice may climb their tail during the TST (Mayorga and Lucki, 2001). When a mouse did so in the current study the operator would gently let it down and continue the testing. Any mouse that climbed its tail more than two times was excluded from the analysis.

2.6. Tissue preparation

The mice were sacrificed 30 min after the last LH training on day 5, and hippocampal tissues were dissected out to determine the BDNF levels after LH training. Coronal slices 400 μm thick were prepared in cold oxygenated artificial cerebrospinal fluid (ACSF)

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