Maternal Separation Induces Different Autophagic Responses in the Hippocampus and Prefrontal Cortex of Adult Rats

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Abstract—Our previous study has reported that maternal separation (MS) results in abnormal behaviors such as depressive-like and anxious-like behaviors in adult rats. However, the underlying mechanism remains unclear. In this study, we focused on the relationship between MS and autophagy in an effort to explain potential mechanisms. Pups experienced MS from postnatal days 7 to 21 and were randomly divided into the control (CON) group and MS group. Data showed that MS induced depressive-like and anxious-like behaviors in adult rats. Compared with the CON group, the glutamate level was obviously increased in the hippocampus (HP) and prefrontal cortex (PFC) of rats in the MS group. Interestingly, both beclin-1 expression and ratio of LC3II/LC3I were decreased in the HP, but they were increased in the PFC. Furthermore, N-methyl-D-aspartate receptor subunit 2B (NR2B) expression was significantly increased in the HP and PFC. Additionally, postsynaptic density-95 was increased in the HP; however, it was unchanged in the PFC. Moreover, the expression of synaptophysin was obviously decreased in both the HP and PFC. In conclusion, these findings suggest that MS induces different autophagic responses in the HP and PFC (i.e., the inhibition of autophagy in the HP and the activation of autophagy in the PFC), which can be related to the NR2B signaling pathway.

Key words: anxiety, autophagy, maternal separation, depression, glutamate.

INTRODUCTION

Recently, some compelling evidence from different studies has demonstrated that adverse childhood experiences (ACEs) not only lead to the impairment of brain structure and function but also induce abnormal behaviors in adults, including depression, anxiety, mania, attention deficit and cognitive dysfunction (Schable et al., 2007; Baek et al., 2012; Leon Rodríguez and Duenas, 2013). ACEs are presented by various forms such as maternal separation (MS), lack of rearing, abuse (physical or sexual) or social isolation (Cicchetti and Manly, 2001; Putnam, 2003; McCrory et al., 2010; Leon Rodriguez and Duenas, 2013). In addition, the incidence of psychopathology in people who experience ACEs is obviously higher than that of people who undergo normal childhood experiences (Cicchetti and Manly, 2001; Putnam, 2003; Leussis et al., 2012). Experimentally, there have been many established animal models of ACEs; the MS model is a well-characterized model for stress (Meaney, 2001). MS could lead to deleterious influence on behavior and physiological responses in animals (Lee et al., 2001; Fenoglio et al., 2006a). Fenoglio et al. reported that good maternal behaviors (e.g. licking or nursing) could be beneficial for the development of the hippocampus (HP), spatial learning and memory in pups (Fenoglio et al., 2006a,b). However, rats that experienced MS had increased apoptosis in the HP and exhibited anxious-like and depressive-like behaviors (Lee et al., 2001; Zhu et al., 2015). The underlying mechanism of MS remains poorly understood and needs to be further investigated.

Autophagy, also termed as type-II programed cell death, maintains cellular homeostasis and is considered an alternative pathway for protein degradation (Kulbe et al., 2014). Autophagy involves removal of impaired organelles and aggregated proteins. Therefore, autophagy is essential for cell survival (Galluzzi et al., 2008; Ma et al., 2012). In addition, autophagy has been considered as having the molecular mechanism of antidepress-
Mammalian separation

The effects of MS on behavioral phenotypes in female mice were inconsistent between different studies (Romeo et al., 2003; Veenema et al., 2007). In addition, estrogen not only has anxiogenic effects but also has anxiolytic effects (Kalandakanond-Thongsong et al., 2012) because of the variation in opposing action on alpha (ERα) and beta (ERβ) estrogen receptors (Newhouse et al., 2010; Fedotova, 2013). To avoid sex-dependent effects, only males were included in our experiment. MS was performed by the method as previously reported in detail (Leon Rodriguez and Duenas, 2013). We designated the day of birth as postpartum day 0 (PPD0). Male pups from each litter were randomly divided into the control group (CON group, n = 10) and the maternal separation group (MS group, n = 10). Pups experienced the separation from their mothers on PPD7. Daily, they were moved onto a heating pad set at 25–28 °C and removed from their home cage for 6 h (3 h in the morning, 7:00–10:00, and 3 h in the afternoon, 13:00–16:00) from PPD7 to PPD21 (Leon Rodriguez and Duenas, 2013). At the same time, pups in the CON group were raised under normal animal facility conditions from PPD7 to PPD21. On PPD22, the rats were weaned. They were reared normally and housed in groups of three per cage until adulthood. Then, they were tested by the OFT, EPM test and FST.

Open-field test

The OFT was performed as previously reported (Harro et al., 1999). Briefly, we put rats into a square box (60 cm × 60 cm × 40 cm) made of wood to carry on the OFT. The floor of the box was divided into 16 squares, and the four central squares were designated as the center of the field. A rat was put in the center of the OFT and allowed to freely move in the field for 5 min. Then, we used a charge-coupled device (CCD) camera to track the movement of the rat, and a computer connected to the CCD camera was applied to record and analyze data (EthoVision 2.0; Noldus, Wageningen, The Netherlands). Finally, we took the rat out and cleaned the field with 70% alcohol to wipe off the rat’s odor. The time spent in the center and the total distance were analyzed because these measures reflected spontaneous activity and anxious-like behavior.

Elevated plus maze test

The EPM test was carried out as previously reported (Bahi et al., 2014). Briefly, the EPM was fixed on a trestle table, which is 40 cm high above the floor. The EPM consists of two closed arms and two open arms, with two

sants. Some antidepressant drugs including maprotiline, fluoxetine and lithium were reported to improve autophagy (Sarkar and Rubinsztein, 2006; Cloonan and Williams, 2011). Rapamycin, a mammalian target of rapamycin (mTOR) inhibitor, also showed antidepressant activity (Cleary et al., 2008). Similarly, Kara et al. also reported that trehalose reduced the immobility of mice in forced swimming tests (FSTs). It suggested that trehalose could ameliorate depressive-like behaviors in mice (Kara et al., 2013).

Furthermore, studies reported that early-life stress is able to enhance glutamate release (O’Connor et al., 2013) and disturb physiological glutamatergic function, which could lead to a hyperglutamatergic state (Musazzi et al., 2011; O’Connor et al., 2013). Interestingly, 1-N-methyl-D-aspartate (NMDA) increases autophagic proteins such as beclin-1 and microtubule-associated protein 1 light chain 3 (LC3) (Perez-Carrion et al., 2012). When beclin-1 is decreased, it could deteriorate NMDA-induced neuronal death. This implies that autophagy is beneficial for rat cortical neuron survival during excitotoxicity (Liu and Zhao, 2013). Conversely, studies reported that excitotoxic glutamate insults blocked autophagic flux in hippocampal neurons (Kulbe et al., 2014). However, little is known about the autophagy presented in MS rats. In this experiment, we employed the well-validated MS rat model; examined the abnormal behaviors of rats by open-field test (OFT), elevated plus maze (EPM) test and FST and aimed to investigate the mechanism of autophagy on MS.

EXPERIMENTAL PROCEDURES

Experimental animals

Adult pregnant female Wistar rats (n = 6) were obtained from the Laboratory Animal Center, Academy of Military Medical Science of People’s Liberation Army, and arrived on day 13 of gestation. All female Wistar rats were reared in standard laboratory cages (26 cm × 42 cm × 15 cm) in a specific pathogen-free house with a 12-h light–dark cycle (i.e., 8 am–8 pm), and they were granted free access to food and water with constant humidity (about 50%) and temperature (21–23 °C). On postpartum day 7, pups from all litters were combined together in one cage and were randomly redistributed (cross-fostered) to litters of 10. Each litter was maintained with a male-to-female ratio of 1:1 and was meant to achieve a balanced distribution for gender. From this moment, the pups and mothers were left undisturbed until the time of separation. This procedure was performed to minimize disruption of the mother–infant relationship (Suchecki et al., 1993). All the procedures were identical to those used previously by other laboratories (Leussis et al., 2012). The Animal Research Ethics Committee of the School of Medicine at Nankai University approved all animal experiments. All animal experiments were performed in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80-23) revised 1996 or the UK Animals (Scientific Procedures) Act 1986 and associated guidelines, the European Council Directive of 24 November 1986 (86/609/EEC) and the Animal Management Rules of the Ministry of Health of the People’s Republic of China. Finally, we ensured that the appropriate pups were used to guarantee confidence of our results in this experiment. This is the precondition using which we try to minimize the total number of animals used and their suffering.

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