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Research Report

Environmental enrichment enhances autophagy signaling in the rat hippocampus

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ABSTRACT

The findings that antidepressive treatments increase hippocampal neurotrophins have led researchers to emphasize the importance of neurogenesis, formation of new dendrites, and survival of neurons in the brain. However, it is difficult to maintain neural plasticity just by enriching the environment to facilitate formation of new networks. Neural plasticity also requires a degradation process that clears off unnecessary and undesirable components. We have recently reported an increase in autophagy signaling (wherein the cell digests components of itself) that has the potential of enhancing neuronal and synaptic plasticity after multiple sessions of electroconvulsive seizure treatment. The present study revealed an increase in autophagy signaling in the rat hippocampus following 2 weeks of environmental enrichment (EE), a procedure known to elicit antidepressive and anxiolytic behavioral changes in various animal paradigms. Western blot analysis showed an increase in hippocampal expression of microtubule-associated protein light chain 3-II (LC3-II), which is lipidated from LC3-I, in rats in the EE group. The effectiveness of the 2-week EE housing condition was validated by anxiolytic effects observed in the elevated plus maze test, enhanced habituation in the open field test, and elevation of hippocampal brain-derived neurotrophic factor expression. In addition, we showed that the EE housing condition ameliorated numbing/avoidance behaviors, but not hypervigilant behaviors, in an animal model of post-traumatic stress disorder (PTSD). This is the first report to show that EE can increase autophagy signaling and improve numbing/avoidance behaviors in an animal model of PTSD.

Abbreviations: AET, avoidance escape task; BDNF, brain-derived neurotrophic factor; CS, conditioned stimulus; ECS, electroconvulsive seizure; ECT, electroconvulsive therapy; EE, environmental enrichment; IP_3, inositol 1,4,5-triphosphate; IS, inescapable foot-shock stress; ITI, inter-trial interval; LC-3, microtubule-associated protein light chain-3; mTOR, mammalian target of rapamycin; PBS, phosphate buffered saline; PI3K, phosphatidylinositol 3-kinase; PTSD, post-traumatic stress disorder; rTMS, repetitive transcranial magnetic stimulation; US, unconditioned stimulus

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1. Introduction

In our previous study (Otabe et al., 2014), we reported an enhancement of hippocampal autophagy signaling following multiple electroconvulsive seizure (ECS) treatments. It has been shown that ECS treatments have neurotrophic (Nibuya et al., 1995), neurogenic (Madsen et al., 2000), morphologically neuroplastic (Vaidya et al., 1999), and anti-depressive (Li et al., 2007) effects in rats. However, stress loading did not result in increased autophagy signaling in the rat hippocampus (Otabe et al., 2014). In the present study, we sought to elucidate the effect of environmentally enriched (EE) housing on hippocampal autophagy signaling by examining the formation of microtubule-associated protein light chain 3-II (LC3-II), which is formed by conjugation of phosphatidylethanolamine at the C terminal region of cytosolic LC3-I and has been used as a biochemical footprint of autophagy (Kabeya et al., 2004). LC3-II is the active form of the protein, which binds to and elongates the autophagosomal membrane prior to fusing with lysosomes to form autolysosomes (Suzuki and Ohsumi, 2007). EE housing is known to exert antidepressive (Brenes Sáenz et al., 2006) and anxiolytic (Fernández-Teruel et al., 1997) behavioral changes. It can also increase learning ability (Nilsson et al., 1999), enhance neurotrophin expression (Ickes et al., 2000), induce neuroplastic morphological changes (Rampon et al., 2000; Turner et al., 2003), and increase hippocampal neurogenesis in rats (Kempermann et al., 2002).

Among the various neurotrophins, brain-derived neurotrophic factor (BDNF) has been most studied in relation to neuronal plasticity elicited by treatment for depression (Duman and Monteggia, 2006). BDNF activates the mammalian target of rapamycin (mTOR), which belongs to Ser/Thr protein kinases. Increased BDNF expression (Takei et al., 2004; Schratt et al., 2004) and potentially antidepressive ketamine treatment (Li et al., 2010) activate mTOR signaling in the local dendrites and contribute to the formation of new neural networks. Although the positive influence of EE housing on hippocampal BDNF expression (Ickes et al., 2000) and anxiety (Fernández-Teruel et al., 1997) has already been reported, we replicated these previous findings in order to validate the 2-week EE housing condition.

Autophagy is a lysosome-dependent degradation mechanism, which clears off and degrades long-lived cytoplasmic proteins or damaged organelles in order to cope with cellular stresses. Such stressors include nutritional starvation at birth (Kuma et al., 2004) and neurodegeneration with protein accumulation in normal cells (Hara et al., 2006). It also occurs in Alzheimer's disease-affected cells (Ma et al., 2010) and tumorigenesis (Mathew et al., 2009). Recent investigations have revealed that activated neurons can induce autophagy-related synaptic remodeling, including the autophagosomal endocytosis of specific receptors on the cell surface (Rowland et al., 2006; Shehata et al., 2012) and synaptic growth and plasticity (Shen and Ganetzky, 2009). Here, we wanted to investigate the autophagy machinery related to synaptic and neuronal plasticity following treatment for depression.

Our working hypothesis is that neuronal plasticity and remodeling would be maintained by the simultaneous stimulation of two opposite directions at different branches even in a single neuron. One direction is the generation and stabilization of new and necessary synaptic connections by activated neurotrophic pathway. The other direction is the erasure of unnecessary synaptic connections and a decrease in the number of specific receptors by autophagy process. Inactivation of mTOR signaling by rapamycin enhances autophagy and is reported to exert antidepressive behavioral changes (Cleary et al., 2008). In addition, the decrease of intracellular inositol and inositol triphosphate (IP-3) concentrations by mood stabilizers is reported to induce the autophagy process through an mTOR-independent pathway. Thus, the effectiveness of mood stabilizers in treating neurodegenerative diseases is gaining much attention (Sarkar et al., 2009).

Currently available medications function on specific neuronal proteins including monoamine transporters, membrane receptors, and metabolic enzymes. However, we believe that studying the fundamental aspects of non-specific but truly effective therapeutic measures like electroconvulsive therapy (ECT), repetitive transcranial magnetic stimulation (rTMS), and EE will help elucidate the vulnerable pathophysiology of stress and depression. In typical EE experiments, animals are housed in conditions aimed at increasing their physical, exploratory, and social activities (van Praag et al., 2000). Although EE is known to increase neurogenesis in dentate granule cell layers (Kempermann et al., 2002), a recent report has suggested that hippocampal neurogenesis by EE has no impact on behavioral changes, including reduced anxiety-like behavior and increased learning ability (Meshi et al., 2006). EE is also reported to increase immune responses (Kingston and Hoffman-Goetz, 1996; Benaroya-Milshtein et al., 2004). We are searching for general and diversely distributed neuroplastic mechanisms induced by ECT, TMS, and EE that do not use chemical psychotropics.

EE treatment has demonstrated therapeutic efficacy in improving cognitive impairment (Jankowsky et al., 2005), mitigating neurodevelopmental disorders including autism (Schneider et al., 2006), reducing anxiety-like behaviors (Roy et al., 2001), shortening freezing time in response to previously loaded stress cues (Benaroya-Milshtein et al., 2004), and in ameliorating depression in animal models (Brenes Sáenz et al., 2006). In the present study, we also explored the therapeutic impact of EE on an animal model of post-traumatic stress disorder (PTSD) that exhibits both numbing/avoidance and hyper-vigilant behaviors (Sawamura et al., 2004; Wakizono et al., 2007; Kikuchi et al., 2008).

2. Results

2.1. *Elevated plus maze test after 2 weeks of EE*

The behavior of 16 control rats (Con) and 12 EE-treated rats (EE) was examined. In both groups, three rats were excluded because they fell from the apparatus. EE-treated rats showed antianxiety effects by increased time spent in the open arms ($6.7 \pm 1.5\%$ in the Con group and $15.7 \pm 3.5\%$ in the EE group; Fig. 2A) and increased entries to open arms ($18.2 \pm 3.8\%$ in the Con group and $36.1 \pm 3.5\%$ in the EE group; Fig. 2B).