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Research Report

Hippocampal signaling pathways are involved in stress-induced impairment of memory formation in rats

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ABSTRACT

Stress is a potent modulator of hippocampal-dependent memory formation. The aim of the present study was to assess the role of hippocampal signaling pathways in stress-induced memory impairment in male Wistar rats. The animals were exposed to acute elevated platform (EP) stress and memory formation was measured by a step-through type passive avoidance task. The results indicated that post-training or pre-test exposure to EP stress impaired memory consolidation or retrieval respectively. Using western blot analysis, it was found that memory retrieval was associated with the increase in the levels of phosphorylated cAMP-responsive element binding protein (P-CREB), peroxisome proliferator-activated receptor gamma coactivator-1α (PGC-1α) and its downstream targets in the hippocampus. In contrast, the stress exposure decreased the hippocampal levels of these proteins. In addition, stress-induced impairment of memory consolidation or retrieval was associated with the decrease in the P-CREB/CREB ratio and the PGC-1α level in the hippocampus. On the other hand, the hippocampal level of nuclear factor E2-related factor 2 (Nrf2) and gamma-glutamylcysteine synthetase (γ-GCS) which are the master regulators of defense system were decreased by the stress exposure. The increased hippocampal levels of Nrf2 and it's downstream was observed during memory retrieval, while stress-induced impairment of memory consolidation or retrieval inhibited this hippocampal signaling pathway. Overall, these findings suggest that down-regulation of

Abbreviations: AD, Alzheimer's disease; APP/PS1, amyloid precursor protein/presenilin 1; ARE, antioxidant responsive element; CAT, activity of catalase; CBP, cAMP-responsive element binding protein (CREB)-binding protein; DTNB, dithionitrobenzoic acid; ECL, electrochemiluminescence; γ-GCS, gamma-glutamylcysteine synthetase; GSH, glutathione; HO-1, heme oxygenase-1; Keap1, Kelch-like ECH-associated protein1; mRNA, messenger ribonucleic acid; NRF-1, neuronal expression of transcription factors including nuclear respiratory factor-1; Nrf2, nuclear factor E2-related factor 2; P-CREB, phosphorylated cAMP-responsive element binding protein; PGC-1α, peroxisome proliferator-activated receptor gamma coactivator-1a; PVDF, poly-vinylidene fluoride membrane; ROS, reactive oxygen species; TFAM, mitochondrial transcription factor A

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CREB/PGC-1α signaling cascade and Nrf2 antioxidant pathways in the hippocampus may be associated with memory impairment induced by stress.

1. Introduction

A large body of evidence suggests that the hippocampus plays a vital role in memory formation via increasing the efficiency of synaptic transmission which is called long-term potentiation (LTP; Pittenger and Kandel). Hippocampal cellular and molecular processes that underlie memory consolidation and retrieval mediate the storage of new information (Whitlock et al., 2006). Hippocampal structure and function have been suggested to be very sensitive to stress (Huang et al., 2005; Han et al., 2013). In addition, while the induction of dendritic branches in the hippocampus is necessary for long-term memory storage (Govindarajan et al., 2011), exposure to stress induces spatial memory deficits through the atrophy of dendrites of hippocampal CA3 neurons (Conrad et al., 1996). It has been shown, using animal learning models, that exposure to acute elevated platform (EP) stress impairs memory consolidation or retrieval (Sardari et al., 2014; Segev et al., 2012). On the other hand, hippocampal LTP induction can be interrupted by different stressors like acute EP (Rocher et al., 2004) or foot shock stress (Xiong et al., 2003).

A variety of signaling pathways which are important in LTP induction including cAMP response element binding protein (CREB) are the main targets of stress (Bilang-Bleuel et al., 2002; St-Pierre et al., 2006). CREB is a transcription factor that activates transcription of target genes by binding to a certain sequence of DNA which are called cAMP response elements (Mizuno et al., 2000). It is well known that long-term memory is dependent on CREB function (Izquierdo et al., 2002; Kogan et al., 2000). It should be noted that upstream signaling pathways such as brain-derived neutrophic factor (BDNF) signaling pathway (Alonso et al., 2002) phosphorylate CREB on a critical serine residue which leads to the activation of this factor (Ying et al., 2002; Lisman et al., 2002). CREB activity madulates the maintenance of LTP and induces memory consolidation of passive avoidance learning (Bernabeu et al., 1997; Barco et al., 2002). Izquierdo et al. (2001) also found that exposure to a novel environment can enhance memory retrieval via increasing the hippocampal CREB phosphorylation. Additionally, some studies have supported a neuroprotective role for CREB which regulates the antioxidant gene expression (Bedogni et al., 2012; Krönke et al., 2003). On the other hand, CREB mediates the activity of peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α) which is a potent stimulator of mitochondrial biogenesis for the formation and maintenance of hippocampal dendritic spines and synapses under environmental challenges (Cheng et al., 2012). PGC-1α stimulates the neuronal expression of transcription factors including nuclear respiratory factor-1 (NRF-1) and mitochondrial transcription factor A (TFAM) in response to stress (Miranda et al., 1999). CREB also interacts with nuclear factor E2-related factor 2 (Nrf2) by CREB binding protein (CBP; Shen et al., 2004a, 2004b). Nrf2 is a heterodimer transcription factor that binds to the antioxidant responsive element (ARE) sequence of DNA, and elevates the level of defense enzymes in the cell (Itoh et al., 1997). Under unstressed conditions, Nrf2 can be anchored in the cytoplasm via binding to Kelch-like ECH-associated protein1 (Tong et al., 2006), while stress exposure leads to the release of Nrf2 from Keap-1 (Dinkova-Kostova et al., 2005) which allows Nrf2 entry into the cell nucleus. There is evidence that enhancing Nrf2 antioxidant signaling pathway improves the learning and memory in animal models (Kanninen et al., 2009). Interestingly, Alzheimer disease decreases the expression levels of NRF -1 and TFAM along with nuclear levels of PGC-1α in the hippocampus, indicating the importance of mitochondrial biogenesis in memory formation (Sheng et al., 2012).

Considering the above-mentioned points and findings, the present study was designed with the following four aims: (i) to determine the effect of exposure to 30 min acute stress in memory consolidation and retrieval of passive avoidance learning; (ii) to investigate the role of P-CREB and its target genes, PGC-1α, NRF-1 and TFAM in the hippocampus during memory formation and stress-induced memory impairment; (iii) to examine the role of Nrf2 and its downstream gamma-glutamylcysteine synthetase (γ-GCS) and heme oxygenase-1 (HO-1); (iiii) to evaluate whether the activation of hippocampal γ-GCS pathway leads the changes of glutathione (GSH) levels and the activity of catalase (CAT; an antioxidant enzyme).

2. Results

2.1. *Effect of post-training or pre-test exposure to acute EP stress on memory retrieval*

Fig. 1 shows the effect of post-training or pre-test exposure to 30 min stress on step-through latency. One-way ANOVA revealed that both post-training and pre-test exposure to acute EP stress reduced the step-through latency in the passive avoidance task [F (2, 18)=42.98, $P<0.001$], indicating stress-induced memory impairment.

2.2. *Changes of hippocampal CREB/PGC-1α signaling cascade in stress-induced memory impairment*

As shown in Fig. 2, the level of P-CREB was measured to assess the possible changes of hippocampal signaling pathways in memory formation under acute EP stress. Since P-CREB directly regulates PGC-1α which is a stimulator for the expression of NRF-1 and TFAM transcription factors (see Section 1), the levels of PGC-1α, NRF-1 and TFAM were also