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HSP70 Facilitates Memory Consolidation of Fear Conditioning through MAPK Pathway in the Hippocampus

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Abstract—Heat shock proteins of the 70-kDa (HSP70) family are cytoprotective molecular chaperones that are present in neuronal cells and can be induced by a variety of homeostatically stressful situations (not only proteostatic insults), but also by synaptic activity, including learning tasks. Physiological stimuli that induce long-term memory formation are also capable of stimulating the synthesis of HSP70 through the activation of heat shock transcription factor-1 (HSF1). In this study, we investigated the influence of HSP70 on fear memory consolidation and MAPK activity. Male rats were trained in contextual fear conditioning task and HSP70 content was analyzed by western blot in the hippocampus at different time points. We observed rapid and transient elevations in HSP70 60 min following training. Double immunofluorescence with GFAP and HSP72 revealed that astrocytes were not the site for HSP72 induction by CFC training. HSP72 distribution markedly surrounded synapses between Shaffer collateral and CA1 pyramidal cells. Infusion of recombinant HSP70 (*hspa1a*) into the dorsal hippocampus immediately after training facilitated memory consolidation and enhanced ERK activity while decreasing the activated forms of JNK and p38 in the hippocampus. Blocking endogenous extracellular HSP70 through the administration of specific antibody did not produce any further effect on memory consolidation when applied immediately after training, suggesting that it is indeed acting intracellularly. Induction of HSP70 after fear conditioning is fast and can act as a signaling molecule, modulating MAPK downstream signaling during memory consolidation in the hippocampus, which is crucial for fear memory formation.

Key words: molecular chaperone, HSP70, contextual fear conditioning, MAPK.

1. INTRODUCTION

Memory consolidation is a process in which new memories are transformed from a labile state to a more stable one. This process depends on the activation of kinases, transcription factors, increased gene expression and protein synthesis in the postsynaptic neuronal cell (Elgersma and Silva, 1999; Abel and Lattal, 2001; Izquierdo et al., 2006). One of the most important pathways for long-term memory formation is the activation of protein kinases by glutamate receptor signaling that leads to the activation of CREB (cAMP responsive element-binding protein), a transcription factor responsible for the activation of different genes involved in memory consolidation (Suzuki et al., 2011; Izquierdo et al., 2006). Among those kinases, the mitogen-activated protein kinase (MAPK) family occupies a critical position. MAPKs are divided into three different subfamilies, including the extracellular signal-regulated kinases (ERK), the c-Jun amino-terminal kinases (JNK) and the p38-MAPK (Seger and Krebs, 1995). JNK was shown to

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Abbreviations: AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; CAMK, Ca^{2+}/calmodulin-dependent protein kinase; CFC, Contextual Fear Conditioning; CNS, central nervous system; CREB, cAMP responsive element-binding protein; ERK, extracellular signal-regulated kinases; GluR, glutamate receptor; HSF-1, heat Shock Factor 1HSP70, heat shock proteins of the 70 kDa; HSP72, inducible form of heat shock protein 70; JNK, c-Jun amino-terminal kinases; LTD, long-term depression; LTP, long-term potentiation; MAPK, mitogen-activated protein kinase; NMDA, N-methyl D-aspartate; p-ERK, phosphorylated ERK; p-JNK, phosphorylated JNK; PKA, protein kinase A; PKC, protein kinase C; rHSP70, recombinant human HSP70.

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be a negative regulator of associative learning and, alongside p38, is involved in synaptic plasticity, inducing long-term depression (LTD) (Moult et al., 2008; Sherrin et al., 2010, 2011). Alternatively, the increase of cAMP and Ca^{2+} levels in the postsynaptic cell enhances both protein kinase A (PKA) and protein kinase C (PKC) activities, respectively, leading to the activation of the ERK pathway, CREB phosphorylation and the initiation of transcription of several genes (Alberini, 2009; Kandel, 2012; Johansen et al., 2011; Roberson et al., 1999). HSP70, a member of the 70-kDa family of heat shock proteins (HSPs), is a potential target gene due to the presence of a CRE motif in its promoter region that can be activated by CREB (Choi et al., 1991; Murshid et al., 2010).

Inducible HSP70 (or HSP72, encoded by *HSPA1A* gene in humans) is a cytoprotective molecular chaperone (Lindquist and Craig, 1988), which is synthesized in the central nervous system (CNS) under a variety of homeostatically stressful situations, including heat shock, glucose and oxygen deprivation, glutamatergic excitotoxicity and psychophysiological stress (Belay and Brown, 2006; Lee et al., 2001). The synthesis of this protein under these conditions protects cells against oxidative stress and cell death, since HSP70 is capable of blocking inflammation and apoptosis signaling (Beere et al., 2000; Garrido et al., 1999). Exogenous HSP70 is able to cross the blood–brain barrier, protect motor neurons from death induced by energy deprivation (Robinson et al., 2005), attenuate seizures (Ekimova et al., 2010) and is envisaged as a potential treatment in Alzheimer's disease, diminishing accumulation of amyloid-β and protecting against spatial memory deficits in animal models of the disease (Bobkova et al., 2014).

In neurons, HSP70 is present in postsynaptic structures (Suzuki et al., 1999) where it can be induced by synaptic activation (Rao and Steward, 1991; Kaneko et al., 1993). Physiological stimuli that induce long-term memory formation, such as increased Ca^{2+}, PKC and Ca^{2+}/calmodulin-dependent protein kinase (CAMK) levels, are also capable of stimulating the synthesis of HSP70 following on from the activation of its most important transcription factor (Heat Shock Factor-1, HSF1) (Price and Calderwood, 1991). Elevation of HSP70 by heat shock prevents the suppression of long-term potentiation (LTP) induced by scopolamine in hippocampal slices (Lin et al., 2004). Similar results have also been observed *in vivo*, in which heat shock pretreatment has shown to block the amnestic effect of scopolamine in the inhibitory avoidance test just 16 h following intervention, a time point of HSP70 peak in the hippocampus (Hung et al., 2004).

Increased HSP70 mRNA and protein content was found to be increased following learning, using different protocols. HSP70 is induced in the hippocampus following aversive and spatial learning (Pizarro et al., 2003; Igaz et al., 2004) and in the cerebellum following a two-way avoidance task (Ambrosini et al., 2005), which suggests that its expression is dependent on the region engaged in the task. Despite numerous assumptions regarding the involvement of HSP70 in synaptic plasticity and memory, there is no concrete evidence of its role and/or downstream signaling in memory formation besides its chaperone function. Therefore, the aim of our study was to verify the influence of HSP70 on memory consolidation and its possible downstream signaling pathways.

2. MATERIALS AND METHODS

Animals

Adult male Wistar rats (270–350 g) from our breeding colony were housed four to five per cage and maintained under constant temperature (23 ± 1 °C) with controlled photoperiods (12 h light/12 h dark; lights on at 7:00 a.m.) and 60% relative humidity. A standard commercial laboratory diet (Nuvilab, Curitiba, Brazil) was provided *ad libitum*. All experiments were performed in accordance with local and national guidelines (Federal Law no 11.794/2008) for animal care and the project was approved by the Ethics Committee on Animal Experimentation of the Federal University of Rio Grande do Sul (CEUA n. 27791).

Stereotaxic surgery and placement of cannulae

Rats were deeply anesthetized via an intraperitoneal injection of ketamine/xylazine (75 and 10 mg/kg, respectively) and bilaterally implanted with 27-gauge guide cannulae with respect to bregma aimed at AP −4.0 mm, ML ± 3.6 mm, DV −1.6 mm (from brain surface), positioned 1.0 mm above the CA1 area of the dorsal hippocampus (Paxinos and Watson, 1998). The animals were exposed to behavioral procedures one week after the surgery. Following behavioral experiments, the rats were euthanized and brains dissected and preserved in 10% formaldehyde to verify cannula position. Only animals with correct cannula placements were included.

Drugs

Recombinant mouse low-endotoxin heat shock protein 70 (hsp72, inducible form of HSP70, encoded by the *hspa1a* gene, Enzo, ADI-ESP-502) was diluted in Dulbecco's PBS pH 7.4, containing 8.1 mM sodium phosphate, 1.5 mM potassium phosphate, 2.7 mM potassium chloride and 137 mM sodium chloride at a total concentration of either 0.25 μg/μL, 0.5 μg/μL or 1.1 μg/μL. Anti-Heat Shock Protein 70 monoclonal antibody produced in mouse (Sigma, H5147, clone BRM–22) was diluted in PBS containing 15 mM sodium azide to a total concentration of 1 μg/μL or 0.1 μg/μL. The vehicle used was the buffer in which the drugs were diluted. Drugs were infused bilaterally into the dorsal hippocampus either immediately, 1 h or 6 h after the training session.

Intrahippocampal infusion

At the time of infusion, a 30-gauge infusion needle was fitted into the guide cannula, with its tip protruding 1.0 mm beyond the end of the guide cannula. A volume of 1μL was infused bilaterally at a slow rate (20 μL/h) and the needle was removed 30 s following complete administration of the drug.

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