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REDUCTION IN HEAT SHOCK PROTEIN 90 CORRELATES TO NEURONAL VULNERABILITY IN THE RAT PIRIFORM CORTEX FOLLOWING STATUS EPILEPTICUS

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Abstract—In the present study, we addressed the question of whether the distinct patterns of heat shock protein (HSP) 70 and HSP90 expressions in the brain region represents the regional specific responses to status epilepsticus (SE) in an effort to better understand the role of HSPs in epileptogenic insult. HSP70 immunoreactivity was increased in CA3 pyramidal cells as well as dentate granule cells at 12 h–1 week after SE. HSP70 immunoreactivity was transiently increased in neurons within the piriform cortex (PC) following SE. Linear regression analysis showed no correlation between the intensity of NeuN and that of HSP70. In contrast to HSP70, HSP90 immunoreactivity was decreased in CA1–3 pyramidal cells at 4 days–4 weeks after SE. In addition, HSP90 immunoreactivity was decreased in PC neurons at 12 h–4 weeks after SE. linear regression analysis showed a direct proportional relationship between the intensity of NeuN and that of HSP90. Therefore, these findings suggest that HSP90 degradation may be closely related to neuronal vulnerability to SE insult.

Key words: HSP70, HSP90, epilepsy, priform cortex, hippocampus, status epilepticus.

INTRODUCTION

Heat shock protein (HSP) is a large family of highly conserved molecules found in all eukaryotic cells (Burdon, 1986). HSP plays a protective role against protein misfolding and aggregation in neurological disorders (Muchowski and Wacker, 2005; Asea and Brown, 2008). HSPs are divided into two broad categories: constitutive HSPs (HSP40 and HSP90), and inducible HSPs (HSP27, HSP32, and HSP70). Interestingly, constitutive HSP and inducible HSP function differently at various physiological conditions. Constitutive HSP serves as a molecular chaperone assisting in the process of protein synthesis required for normal cell growth and metabolism under unstressed conditions. In contrast, inducible HSP functions as a stress protein during stress conditions. Under stress conditions, inducible HSP expression temporarily increases to assist cellular recovery by enhancing the ability of the cell to cope with increased concentrations of unfolded/denatured proteins (Pelham, 1984; Nollen et al., 1999; Buccellato et al., 2007).

Pilocarpine, a cholinergic agonist, induces status epilepticus (SE) in rodents. Pilocarpine-induced SE shows massive neuronal loss in the hippocampus followed by glial proliferation (Kang et al., 2006; Kim et al., 2010a,b, 2011b, 2012, 2013). This neuronal damage in the pilocarpine model is not restricted to the hippocampus, but often extends to extrahippocampal limbic structures. Although neuronal damage caused by SE is characterized by an increased expressions of HSP70 and HSP27 (Akbar et al., 2001), the role of the HSP in neurodegeneration during epilepsy still remains uncertain. Yang et al. (2008) reported that HSP70 was a useful indicator of stressed neurons in acute phase of epilepsy induced by kainate, but not associated with neuronal death, thereby suggesting that HSP70 played no role in neuroprotection during an epileptogenic state. In contrast, Rejdak et al. (2012) suggested that HSP70 induction is of potential value as sensitive and specific biomarkers of seizure-related brain pathologic events. Furthermore, information on expression patterns of HSP90 in the brain following SE has been limited, although the expression level of HSP90 shows little change in the rat hippocampus following pilocarpine-induced SE (Lively and Brown, 2008). With respect to the differential profiles of neuronal death induced by SE (Ryu et al., 2010; Kim et al., 2011a), therefore, it is noteworthy that the specific pattern of HSP expression would contribute to neuronal vulnerability in response to SE. Therefore, during the course of this study, we addressed the question of whether the distinct patterns of HSP70 and HSP90 in the brain region represents the regional specific responses to SE insult in an effort to better understand the role of HSPs in epileptogenic insult.

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Abbreviations: EDTA, ethylenediaminetetraacetic acid; FJB, Fluoro-Jade B; HSF1, heat shock factor 1; HSP, heat shock protein; PB, phosphate buffer; PBS, phosphate-buffered saline; PC, piriform cortex; SE, status epilepticus.

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EXPERIMENTAL PROCEDURES

Experimental animals and chemicals

This study utilized a progeny of Sprague–Dawley (SD) rats (male, 9–11 weeks old) obtained from Experimental Animal Center, Hallym University, Chunchon, South Korea. The animals were provided with a commercial diet and water *ad libitum* under controlled temperature, humidity and lighting conditions (22 ± 2 °C, 55 ± 5% and a 12:12 light/dark cycle with lights). Animal protocols were approved by the Institutional Animal Care and Use Committee of the Hallym University. Procedures involving animals and their care were conducted in accord with our institutional guidelines that comply with NIH Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80-23, 1996). In addition, we have made all efforts to minimize the number of animals used and their suffering.

Seizure induction

Animals were given LiCl (Sigma–Aldrich Co., St. Louis, MO, USA; 127 mg/kg i.p.) 20 h before the pilocarpine treatment. Animals were treated with pilocarpine (Sigma–Aldrich Co., St. Louis, MO, USA; 25 mg/kg, i.p.) 30 min after scopolamine butylbromide (Sigma–Aldrich Co., St. Louis, MO, USA; 2 mg/kg, i.p.). Approximately 80% of pilocarpine-treated rats showed acute behavioral features of SE (including akinesia, facial automatisms, limbic seizures consisting of forelimb clonus with rearing, salivation, masticatory jaw movements, and falling). Diazepam (10 mg/kg, i.p.) was administered 2 h after the onset of SE and repeated as needed. One week after SE, rats were observed 3–4 h a day in the vivarium for general behavior and occurrence of spontaneous seizures. The onset of spontaneous seizure occurrence was 3–4 weeks after SE. On average, these animals developed two seizures during observation (3–4 h/day). Age-matched animals were used as non-SE experienced controls (non-SE animals). Non-SE animals received saline in place of pilocarpine.

Tissue processing

At designated time points (non-SE, 12 h, 1 day, 2 days, 3 days, 4 days, 1 week and 4 weeks after SE; $n = 5$, for each time point), animals were perfused transcardially with phosphate-buffered saline (PBS) followed by 4% paraformaldehyde in 0.1 M phosphate buffer (PB, pH 7.4) under urethane anesthesia (Sigma–Aldrich Co., St. Louis, MO, USA; 1.5 g/kg, i.p.). The brains were removed, and postfixed in the same fixative for 4 h. The brain tissues were cryoprotected by infiltration with 30% sucrose overnight. Thereafter, the brain was frozen and sectioned with a cryostat at 30 μm and consecutive sections were contained in six-well plates containing PBS. For stereological study, every sixth section in the series throughout the entire hippocampus and piriform cortex (PC) were used in some animals. Some sections were mounted on gelatin-coated slides, and then stained with Nissl (Cresyl violet) to assess neuronal damage (Kang et al., 2006).

Immunohistochemistry

Free-floating sections were first incubated with 10% normal chicken serum (Vector, Burlingame, CA, USA) for 30 min at room temperature. They were then incubated in rabbit anti-HSP70 IgG (Enzo Life Science, Farmingdale, NY, USA; diluted 1:200) or rabbit anti-HSP90B1 IgG (Proteintech, IL, USA; diluted 1:200) in PBS containing 0.3% Triton X-100 (Sigma–Aldrich Co., St. Louis, MO, USA) and 2% normal chicken serum (Sigma–Aldrich Co., St. Louis, MO, USA) overnight at room temperature. After washing three times for 10 min with PBS, the sections were incubated sequentially, in goat anti-rabbit IgG (Vector, Burlingame, CA, USA; diluted 1:250) and ABC complex (Vector, Burlingame, CA, USA), diluted 1:200 in the same solution as the primary antiserum. Between the incubations, the tissues were washed with PBS three times for 10 min each. The sections were visualized with 3,3′-diaminobenzidine (DAB, Sigma–Aldrich Co., St. Louis, MO, USA) in 0.1 M Tris buffer and mounted on the gelatin-coated slides. For negative controls, tissues were incubated with 1 μg of the antibody that was preincubated with 1 μg of purified peptide (HSP70 and HSP90B1) for 1 h at room temperature or incubated with pre-immune serum instead of the primary antibody (NeuN). All images were captured using an AxioImage M2 microscope and AxioVision Rel. 4.8 software.

Double immunofluorescence study

Sections were incubated with 3% bovine serum albumin in PBS for 30 min at room temperature. Sections were then incubated in a mixture of mouse anti-NeuN IgG (a neuronal marker, Millipore Corporation, Billerica, MA, USA; diluted 1:500)/rabbit anti-HSP70 IgG (Enzo Life Science, IL, USA; diluted 1:100) or rabbit anti-HSP90B1 IgG (Ptglab, IL, USA; diluted 1:100) in PBS containing 0.3% triton X-100 overnight at room temperature. After washing three times for 10 min with PBS, sections were also incubated in a mixture of FITC-conjugated secondary antiserum (or FITC-conjugated streptavidin for isolectin B4) and Cy3-conjugated secondary antisera (Amersham, San Francisco, CA, USA), diluted 1:200, for 2 h at room temperature. The sections were washed three times for 10 min with PBS, and mounted on gelatin-coated slides. For nuclei counterstaining, we used Vectashield mounting medium with DAPI (Vector, Burlingame, CA, USA). All images were captured using an AxioImage M2 microscope and AxioVision Rel. 4.8 software.

Fluoro-Jade B (FJB) staining

FJB staining was used to identify degenerating neurons in every group. Briefly, sections were rinsed in distilled water, and mounted onto gelatin-coated slides and then dried on a slide warmer. The slides were immersed in