Published in final edited form as:

Neurosci Lett. 2010 March 19; 472(2): 90–93. doi:10.1016/j.neulet.2010.01.049.

Age-related changes in HSP25 expression in basal ganglia and cortex of F344/BN rats

Anisha A. Gupte¹, Jill K. Morris¹, Hongyu Zhang¹, Gregory L. Bomhoff¹, Paige C. Geiger^{1,2,3}, and John A. Stanford^{1,2,3,*}

¹Department of Molecular and Integrative Physiology, University of Kansas Medical Center, Kansas City, KS, 66160

²Smith Intellectual & Developmental Disabilities Research Center, University of Kansas Medical Center, Kansas City, KS, 66160

³Landon Center on Aging, University of Kansas Medical Center, Kansas City, KS, 66160

Abstract

Normal aging is associated with chronic oxidative stress. In the basal ganglia, oxidative stress may contribute to the increased risk of Parkinson's disease in the elderly. Neurons are thought to actively utilize compensatory defense mechanisms, such as heat shock proteins (HSPs), to protect from persisting stress. Despite their protective role, little is known about HSP expression in the aging basal ganglia. The purpose of this study was to examine HSP expression in striatum, substantia nigra, globus pallidus and cortex in 6-, 18- and 30-month-old Fischer 344/Brown Norway rats. We found robust age-related increases in phosphorylated and total HSP25 in each brain region studied. Conversely, HSP72 (the inducible form of HSP70) was reduced with age, but only in the striatum. p38 MAPK, a protein implicated in activating HSP25, did not change with age, nor did HSC70 (the constitutive form of HSP70), or HSP60. These results suggest that HSP25 is especially responsive to age-related stress in the basal ganglia.

Keywords

heat shock; basal ganglia; aging; Parkinsonism; neuroprotection; chaperones; stress

Normal aging is associated with oxidative stress and mitochondrial dysfunction. In the brain, these changes are thought to contribute to age-related diseases such as Parkinson's disease (PD) and Alzheimer's disease (AD) [1]. One mechanism by which neurons respond to increased cellular stress is by inducing the expression of heat shock proteins (HSPs) [3]. HSP70 exhibits neuroprotective effects in models of PD, AD and amyotrophic lateral sclerosis (ALS) [2]. HSC70, the constitutively expressed member of the HSP70 family, and HSP72, the inducible form of HSP70 have been found to increase with age in the brain [3,19]. Although the smaller HSP25 has been reported to exhibit neuroprotective properties [5,14], its expression has not been characterized in the aging brain. Because of our interest in age-related changes in basal

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*Correspondence to: John A. Stanford, Ph.D. Department of Molecular & Integrative Physiology University of Kansas Medical Center 3901 Rainbow Blvd., MS 3051 Kansas City, KS 66160 Phone: 913-588-7416 Fax: 913-588-5677 jstanford@kumc.edu.

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ganglia and motor function [16,17,21], we measured several HSPs in the basal ganglia and cortex of aging rats in order to determine whether their expression changes with age.

Male F344/BN rats were purchased at 6 (n=3, body weight 466.67 ± 5.77 g), 18 (n=3, body weight 518.67 ± 32.02 g) and 30 (n=3, body weight 625 ± 43.00 g) months of age from NIA colonies. Rats were housed in pairs in large cages in a temperature controlled facility with a 12 h light/dark cycle, with *ad libitum* access to food and water. Rats were anesthetized with sodium pentobarbital, their brains were removed, and the following sections were collected in tubes and frozen on dry ice: striatum, substantia nigra, globus pallidus and cortex. Protocols were approved by the KUMC Institutional Animal Care and Use Committee and procedures adhered to the *Guide for the Care and Use of Laboratory Animals*.

Antibodies against HSP72 (SPA-810), phospho-HSP25 (Ser 82), total HSP25, HSC70 (SPA-815) and HSP60 were obtained from Stressgen (Victoria, BC, Canada), and antibodies against Actin were obtained from Abcam (Cambridge, MA). Antibodies against phospho-p38 MAPK (Thr 180/Tyr 182), total p38 MAPK, phospho-SAPK/JNK (T183/Y185), and total SAPK/JNK were purchased from Cell Signaling (Beverly, MA). Goat-anti-rabbit HRP-conjugated secondary antibodies were obtained from Santa Cruz Biotechnology (Santa Cruz, CA), while goat anti-mouse HRP-conjugated secondary antibodies were obtained from Bio-Rad (Hercules, CA). Enhanced chemiluminescence reagents were purchased from Amersham (Little Chalfont, Buckinghamshire, UK). All other reagents were obtained from Sigma (St.Louis, MO).

Frozen brain sections were homogenized using a hand held homogenizer in a 12:1 (volume to weight) ratio of ice cold buffer from Biosource (Invitrogen, Carlsbad, CA) as described before [12]. Homogenized samples were rotated for 30 minutes and then centrifuged for 30 minutes at 3,000 rpm at 4°C. The protein concentration of the supernatant was determined by Bradford method (Bio-Rad, Hercules, CA). Samples were prepared in 1X Laemmli buffer (final) containing 100 mM dithiothreitol and denatured in a boiling water bath for 5 minutes. Protein samples were subjected to SDS-PAGE and Western blots were performed as described before [12]. One-way analysis of variance was used to evaluate differences between age groups within each brain section. This was followed by a post hoc comparison using the Student-Newman-Keuls test when appropriate. Statistical significance was set at $P < 0.05$.

Robust increases in phosphorylated HSP25 (pHSP25) occurred with age in striatum, substantia nigra, globus pallidus and cortex sections (Figure 1A-D). The greatest increase in pHSP25 was in the GP, as 30-month-old animals exhibited levels 1356% greater than 6-month-olds. While pHSP25 peaked at 30 months in each brain section examined, pHSP25 was increased in the middle-aged group only in the SN. Total HSP25 expression also increased markedly in all 4 brain sections in the 30-month-old animals compared to the 6-month-old animals. Like pHSP25, HSP25 was greatest in the GP of 30-month-old animals.

HSP72, the inducible form of HSP70, was examined using an antibody that specifically recognizes HSP72 and does not cross-react with HSC70. Although HSP72 expression was very low compared to HSC70, the striatum showed a significant decrease with age (Table 1). Compared to the 6-month-old group, HSP72 expression was 42% less in the 18-month-old animals and 49% less in the 30-month-old animals. The substantia nigra, globus pallidus and cortex did not show significant changes in HSP72 expression in either age group. HSC70, the constitutively expressed HSP70 family member, was examined using an antibody that specifically reacts with HSC70 and does not cross react with HSP72. HSC70 was abundantly expressed in each brain region, but did not vary significantly with age (Table 1).

Impaired mitochondrial function is associated with age-related neurodegenerative diseases. Because HSP60 is associated with the mitochondrial heat shock response, we quantified its

expression. HSP60 expression did not change with age in any of the brain sections examined (Table 1). In addition, we analyzed activation of c-Jun N-terminal kinase (JNK). Stress kinases, such as JNK, are known to be activated by phosphorylation in conditions of oxidative stress. Although we observed an age-related increase in JNK phosphorylation (i.e., pJNK; Table 1), this trend did not reach significance.

In this study we report that HSP25 is markedly upregulated in striatum, substantia nigra, cortex and globus pallidus, while HSP72 is decreased in the striatum of aging F344/BN rats. Increased HSP25 has been reported in brainstem motor nuclei of mice following heat stress and hypoxia [13], and there is evidence under various conditions that this upregulation is protective. In PC12 cells, HSP25 inhibits 6-hydroxydopamine-induced cytochrome c release and apoptosis [9], and protects complex I activity during heat and oxidative stress [5]. HSP25 upregulation in the spinal cord of a mouse model of ALS is accompanied by increased levels of the antioxidant peroxiredoxin 6 [18], and neurodegeneration has been reported to occur in this model only after HSP25 compensation fails [11]. Thus, increased HSP25 appears to be a defense mechanism for neuronal cells under conditions of stress.

One protein implicated in activating HSP25 is p38 MAPK. While there was a trend toward increased total p38 MAPK with age, this increase did not reach significance in any of the brain sections examined (Table 1). The p38 MAPK has been suggested to be the primary upstream kinase of HSP25, phosphorylating and activating HSP25 in response to stress [20]. Phosphorylation of p38 MAPK indicates increased activity. The fact that we saw robust increases in phosphorylated HSP25 but no significant changes in p38 MAPK activation suggests that phosphorylation of HSP25 in the aging brain is mediated by other kinases, such as PKD, PKD2 and ERK [7,20]. On the other hand, because of the trend toward increased p38 MAPK, a role for this protein in HSP25 activation cannot be ruled out. Further studies are clearly warranted to investigate the role of these kinases in HSP25 activation.

Although others have reported increased HSC70 in the brains of aging rats [3,19], we saw decreases in the inducible HSP72 with age in the striatum. Our finding is consistent with reports of decreased HSP expression in skeletal and cardiac muscles with age [4,10]. It is possible that strain differences may have played a role in the discrepancy between our findings using F344/BN rats and previous findings using Wistar rats. For example, there are substantial differences in lifespan between Wistar rats and rats with a Brown Norway background [6,15]. It may be that the diminished HSP72 that we observed in the striatum in our study relates to the greater longevity in the F344/BN strain. Nevertheless, while findings regarding the effects of normal aging on HSP70 in the brain remain equivocal, HSP25 appears to be especially responsive to aging in the basal ganglia. Maintaining or upregulating HSP expression may be an important strategy for preventing age-associated neurodegenerative diseases.

Acknowledgments

The authors would like to thank Michele Healy for assistance with this manuscript. There are no actual or potential conflicts of interest including any financial, personal or other relationships with other people or organizations within three years of beginning the work submitted that could inappropriately influence this work. Animal protocols were approved by the KUMC IACUC. This study was supported by NIH grants AG023549, AG026491 and the Kansas City Area Life Sciences Institute.

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Figure 1. Increased expression of phospho-HSP25 and total HSP25 with age in the basal ganglia and cortex

Expression levels of p-HSP25 and total HSP25 were measured with Western blot analysis in the striatum (A), substantia nigra (B), globus pallidus (C) and cortex (D) of 6-month-old, 18-month-old and 30-month-old rats. All blots were normalized to actin expression as a loading control. (***) $P < 0.001$, (**) $P < 0.01$, (*) $P < 0.05$. Values are means $\pm$ SE for 3 rats per group.

Table 1

Expression of HSC70, HSP72, HSP60, phosphorylated p38 MAPK, total p38 MAPK, phosphorylated JNK, and total JNK was analyzed in the basal ganglia and cortex of 6-, 18- and 30-month-old F344/BN rats. Values are means $\pm$ SE for 3 rats per group.

	Striatum			Substantia Nigra		
	6 mos	18 mos	30 mos	6 mos	18 mos	30 mos
HSC70	0.414 $\pm$ 0.06	0.496 $\pm$ 0.05	0.435 $\pm$ 0.05	0.459 $\pm$ 0.02	0.471 $\pm$ 0.02	0.442 $\pm$ 0.04
HSP72	0.175 $\pm$ 0.11	0.067 $\pm$ 0.05*	0.068 $\pm$ 0.02*	0.287 $\pm$ 0.01	0.340 $\pm$ 0.05	0.335 $\pm$ 0.03
HSP60	0.665 $\pm$ 0.06	0.726 $\pm$ 0.06	0.746 $\pm$ 0.11	0.511 $\pm$ 0.05	0.539 $\pm$ 0.08	0.587 $\pm$ 0.04
p-p38 MAPK	0.111 $\pm$ 0.05	0.139 $\pm$ 0.04	0.192 $\pm$ 0.03	0.170 $\pm$ 0.04	0.268 $\pm$ 0.08	0.285 $\pm$ 0.03
p38 MAPK	0.411 $\pm$ 0.11	0.544 $\pm$ 0.03	0.488 $\pm$ 0.06	0.258 $\pm$ 0.03	0.299 $\pm$ 0.04	0.308 $\pm$ 0.19
pJNK	0.362 $\pm$ 0.09	0.452 $\pm$ 0.02	0.466 $\pm$ 0.005	1.535 $\pm$ 0.15	2.480 $\pm$ 0.90	2.820 $\pm$ 0.26
	Globus Pallidus			Cortex		
	6 mos	18 mos	30 mos	6 mos	18 mos	30 mos
HSC70	0.588 $\pm$ 0.06	0.618 $\pm$ 0.08	0.600 $\pm$ 0.04	0.817 $\pm$ 0.05	0.741 $\pm$ 0.08	0.755 $\pm$ 0.03
HSP72	0.365 $\pm$ 0.04	0.400 $\pm$ 0.05	0.370 $\pm$ 0.07	0.432 $\pm$ 0.04	0.360 $\pm$ 0.02	0.382 $\pm$ 0.03
HSP60	0.417 $\pm$ 0.07	0.388 $\pm$ 0.08	0.422 $\pm$ 0.03	0.656 $\pm$ 0.02	0.693 $\pm$ 0.14	0.594 $\pm$ 0.05
p-p38 MAPK	0.145 $\pm$ 0.02	0.150 $\pm$ 0.01	0.188 $\pm$ 0.03	0.139 $\pm$ 0.02	0.131 $\pm$ 0.03	0.214 $\pm$ 0.04
p38 MAPK	0.272 $\pm$ 0.06	0.322 $\pm$ 0.03	0.421 $\pm$ 0.06	0.323 $\pm$ 0.03	0.318 $\pm$ 0.04	0.393 $\pm$ 0.02
pJNK	1.382 $\pm$ 0.09	1.475 $\pm$ 0.43	2.142 $\pm$ 1.06	0.935 $\pm$ 0.07	0.957 $\pm$ 0.04	1.085 $\pm$ 0.09

Note:

* p<0.05 compared to 6-month-old group.