Age-Related Changes in Heat Shock Protein Expression in the Mouse Hippocampus

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Abstract
Heat shock proteins (Hsps) are a class of molecules inside the cells of the body which function to stabilize and protect damaged or misfolded proteins and prevent their aggregation. Hsps are expressed in cells at basal levels necessary to deal with the daily stressors that result in the formation of toxic misfolded proteins. In addition, the expression level of many Hsps is elevated in response to additional stress to the cell in order to protect against an increase in toxic aggregates. In the brain, these toxic aggregates may cause neuronal cell death, loss of cognitive function, and a number of neurodegenerative diseases such as Alzheimer’s disease (AD). One of the first areas of the brain to be affected in AD is the hippocampus. It has been proposed that aged animals lose the ability to regulate the level of expression of Hsps in order to deal with the onslaught of cellular stress, and that this deregulation may contribute to the onset and progression of AD. We hypothesize that aged animals have elevated levels of constitutive Hsps due to the increase in cellular stress associated with aging. We further hypothesize that aged animals will demonstrate a reduced ability to increase expression of inducible Hsps in response to additional stress. In order to compare the levels of constitutive and inducible Hsps, we induced hyperthermia in three age groups of mice. We compared hippocampal levels of four Hsps previously shown to stabilize and protect two proteins commonly associated with AD, amyloid beta and tau. Results are currently being compiled and will be available on the poster.

Introduction
- Heat Shock Proteins (Hsps) are involved in the maintenance and stabilization of proteins that have become damaged of misfolded.
- When damaged or misfolded proteins aggregate in the brain, they form plaques that are toxic to neurons and eventually lead to cell death and loss of cognitive function.
- Aβ and tau are the two main proteins involved in AD pathology, both of which are known to be stabilized by Hsps.
- The hippocampus is one of the first areas to be affected by AD. This area of the brain is imperative in learning and spatial memory.
- As humans age, there is a greater incidence of damaged and misfolded proteins leading to plaque formation. This can lead to a multitude of neurodegenerative diseases such as Alzheimer’s Disease (AD) and Parkinson's Disease.
- This study aims to compare the baseline and inducible levels of Hsps in three age groups of mice.

Methods

Test Subjects: The young group consisted of mice ranging from 4-6 weeks of age (n=15). The middle aged group ranged from 8-10 months (n=15), and the old group ranged from 18-24 months (n=15).

Heat Shock: To induce a stress response, the mice were anesthetized with ketamine and xylazine and heated under a heat lamp to a colonic temperature of 39°C, or 5°C over basal colonic temperature, as measured by a rectal thermometer. This temperature was maintained for 15 minutes before the mice were allowed to cool and were placed back into their home cages.

Tissue Removal: 24 hours after the heat shock, the mice were euthanized by CO2 asphyxiation, brains removed, and hippocampus dissected.

Analysis: The removed tissue was then lysed in lysis buffer and the Hsp proteins were visualized by Western Blot. Western blot results were analyzed using computer densitometry.

Results

Figure 1. Baseline and inducible Hsp expression compared across three age groups. A. There is an increase in baseline HSP70 expression with increased age, though not significant. B. As age increases, the ability to induce HSP70 expression increases significantly. C. There is increased baseline expression of HSP105 in middle aged animals when compared to young animals, though not significantly. Old animals showed lower baseline HSP105 expression when compared to young mice, but again this difference was not significant. D. Young and old mice showed a strong ability to induce HSP105 expression when compared to middle aged animals, but only young mice showed a significant induction of HSP105. E. Baseline expression of CHIP significantly increased with age however there was no difference (P) in ability to induce a response to heat shock. G. There is significantly decrease in constitutive HSC70 expression with age, but there was no difference in ability to induce HSC70 in response to heat shock (H).

Conclusions
HSP70: Due to chronic cellular stress of aging, baseline HSP70 expression increases with age, though not significantly. Interestingly, there is a trend toward lower expression in older mice when compared to young mice (Figure 1C). Middle-aged mice showed a reduced ability to induce HSP105 expression following heat shock when compared to old mice and young mice (Figure 1D).

CHIP: Similarly to HSP70 (Figure 1A), the chronic cellular stress of aging causes baseline expression of CHIP to significantly increase with age (Figure 1E). However, all three age groups showed a similar ability to induce expression following heat shock (Figure 1F).

HSC70: Baseline expression of constitutive HSC70 significantly decreased with age (Figure 1G). Following heat shock, all three age groups showed a similar HSC70 response (Figure 1H).

Discussion
Aged mice showed significantly higher baseline expression of HSP70 (Figure 1A) and CHIP (Figure 1E) due to the chronic cellular stress of aging. It is likely that because of the higher baseline expression of these two HSP’s, old animals are unable to further induce HSP70 (Figure 1B) and CHIP (Figure 1F) expression in response to acute stress. It is interesting that HSC70 is generally regarded as the constitutively expressed Hsp and our findings suggest that the aged animal has less of this Hsp with which to protect the hippocampus from cellular stress. HSP70 is generally considered the important “inducible” gene in this family and our data suggests that older animal have lost the ability to upregulate this expression of this gene in response to cellular stress. CHIP is a protein that interacts with HSP70, and thus it is not surprising that its level of expression increases with that of HSP70 as the animal ages. What is surprising, however, is that the level of CHIP does not change in response to our heat shock protocol, suggesting that expression of CHIP and HSP70 may be differentially regulated. The aged animals reduced ability to induce an HSP70 and the lack of a CHIP response may correlate with the increased incidence of protein-related neurodegenerative diseases such as Alzheimer’s disease.