CHAPTER 12

HEAT SHOCK PROTEINS AT THE SYNAPSE: IMPLICATIONS FOR FUNCTIONAL PROTECTION OF THE NERVOUS SYSTEM

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Abstract: The protective effects of prior heat shock against cell death have been well established. Comparatively little attention has been given to the determination of whether this type of 'preconditioning' treatment protects critical neural processes such as synaptic function from subsequent stress. Synapses are key sites of information transfer in the nervous system and their functionality must be preserved under stressful conditions to prevent communication breakdown. Synaptic connections are vulnerable regions of neurons involved in the physiological process of 'neurotransmission' that link neurons into functional networks. The combined application of molecular biology and neurophysiology techniques has demonstrated that prior heat shock protects neurotransmission and synapses are able to function under conditions that would normally be disruptive. Selective overexpression of Hsp70 enhances the level of synaptic protection. Biochemical isolation of synaptic fractions and immunocytochemistry has localized a set of constitutive and stress-inducible heat shock proteins to components of the synapse. Constitutively expressed Hsc70 protein is enriched in neural tissue compared to non-neural tissues. Following hyperthermia, an enhancement of Hsc70 is apparent in synapse-rich areas of the brain in concert with the appearance of stress-inducible Hsp70, Hsp32 and Hsp27 at synapses. Induction of the heat shock response protects the nervous system at the functional level and permits neurotransmission events to proceed at synapses during stressful conditions. Synaptic function is disrupted during the progression of neurodegenerative diseases and upregulation of heat shock proteins could mitigate that dysfunction

Keywords: Heat shock response; heat shock proteins; synapses; neurotransmission; neuroprotection; neurodegenerative diseases

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INTRODUCTION

In response to adverse stimuli, cells activate a highly conserved 'heat shock response' in which a set of stress proteins, termed 'heat shock proteins' (Hsps), are induced. These proteins play important roles in cellular repair and protective mechanisms. Induction of the heat shock response protects cells from subsequent stress that would normally be lethal. This phenomenon, termed 'induced thermotolerance', was initially investigated in tissue culture systems. However, the ability of the heat shock response to protect against cell death induced by a range of stressful stimuli has also been demonstrated in the intact nervous system (reviewed by Brown, 1994, 2007; Brown and Sharp, 1999; Franklin et al., 2005). Comparatively little progress has been made regarding the mechanisms by which the heat shock response protects cells at a functional level. Are there important physiological processes unique to the nervous system that must be protected during stressful conditions? Synapses are critical points of information transfer between neurons. Their functionality must be preserved during stressful conditions to prevent communication breakdown in the nervous system. Synaptic connections are vulnerable regions of neurons involved in the physiological process of 'neurotransmission' that functionally link neurons into communication networks. This review explores whether induction of the heat shock response protects neurons at the functional level of the synapse and whether this strategy could impact synaptic dysfunction that is a feature of neurodegenerative diseases.

THE HEAT SHOCK RESPONSE IN THE NERVOUS SYSTEM

The classic heat shock response was initially studied in non-neural tissue culture systems. This provided the impetus for subsequent investigations that demonstrated that the response is physiologically relevant as it could be induced in the mammalian nervous system following a fever-like increase in body temperature or tissue injury (Brown, 1994, 2007; Brown and Sharp, 1999; Franklin et al., 2005). A robust induction of Hsp70, Hsp32 and Hsp27 is triggered in brain cells following hyperthermia (Manzerra et al., 1993, 1997; Foster and Brown, 1997; Bechtold and Brown, 2000; Bechtold et al., 2000; Franklin et al., 2005).

Stressful stimuli can elicit two reactive responses: the heat shock response and activation of the cell death pathway. Most studies on the in vivo effects of hyperthermia on mammalian tissues have focused on the induction of Hsps. However, a fever-like increase in body temperature has been shown to induce cell death in dividing cell populations of the testis and thymus of the adult rat but not in mature, postmitotic cells of the brain (Khan and Brown, 2002). Hyperthermia did induce cell death in brain cells at early stages in development when neural cells undergoing cell division are present. These results suggest that actively dividing cell populations in vivo are more prone to cell death induced by hyperthermia than fully differentiated, postmitotic neural cells (Khan and Brown, 2002). Correlation of the patterns of cell death and Hsp70 expression revealed that cells inducing Hsp70 after whole

body hyperthermia were not triggered into cell death (Belay and Brown, 2003). Interestingly, populations of neurons in the adult brain rat, such as hippocampal neurons and Purkinje neurons, did not induce Hsp70 after hyperthermia and were not triggered into cell death (Belay and Brown, 2006). High expression of constitutively expressed Hsc70 was noted in these neuronal cell populations, suggesting that neuronal expression of Hsc70 may play roles in 'preprotecting' neurons from stressful stimuli (Belay and Brown, 2006).

PRIOR HEAT SHOCK PROTECTS THE NERVOUS SYSTEM AT THE FUNCTIONAL LEVEL OF THE SYNAPSE

Prior exposure to sublethal temperatures induces heat shock proteins and protects cells from death that would normally result from exposure to lethal temperatures and other forms of stress (Morimoto et al., 1997). The nervous system follows this general rule as induction of the heat shock response by 'preconditioning' protects the nervous system against subsequent insults (Brown, 1994; Yenari, 2002). Protective effects of prior heat shock treatment have been reported in both neural cells grown in tissue culture and in the intact nervous system. For example, prior heat shock at 42°C protects rat embryos from developmental neural defects caused by heat shock at 43°C (Walsh et al., 1987, 1989). Prior heat shock also protects retinal photoreceptors from cell death induced by bright light (Barbe et al., 1988). The time course of Hsp70 induction parallels the time course of the protective effect and intraocular injection of exogenous Hsp70 reduces the susceptibility of the retinal cells to light damage (Tytell et al., 1993; Yu et al., 2001).

The protective effects of prior heat shock treatment against cell death have been well established, but comparatively little is known with respect to whether this type of 'preconditioning' protects critical neural processes such as synaptic function from subsequent stress. Synapses are critical sites of information transfer in the nervous system and their functionality must be maintained under stressful conditions to prevent communication breakdown. Synapses are vulnerable regions of neurons involved in the physiological process of 'neurotransmission' that link neurons into functional networks. Exploration of the neural heat shock response at the level of the synapse requires the combined application of molecular and cellular techniques to measure induction of Hsps and neurophysiological techniques to measure neurotransmission events at synapses. Drosophila is an organism that is highly appropriate for studies that combine molecular and cellular biology with neurophysiology.

Interestingly the heat shock response was first described in Drosophila (Ritossa, 1962) and much of the early work on Hsps was carried out on this organism. In Drosophila, synthesis of most cellular proteins is down-regulated during thermal stress while the predominant Hsp70 is rapidly induced and plays a major role in protective mechanisms (Tissieres et al., 1974; Parsell and Lindquist, 1993; Feder et al., 1996). The Drosophila system lends itself to genetic

and molecular manipulation, but it is also ideal for studies on synaptic neurophysiology as the large size and accessibility of the larval neuromuscular junction makes it the premier experimental preparation for investigation of synaptic transmission (Keshishian et al., 1996). A macropatch electrode can be positioned over individual, visualized synaptic 'boutons' to facilitate neurophysiological recordings of pre- and postsynaptic events that modulate synaptic transmission (Cooper et al., 1995; Stewart et al., 1996). Drosophila larvae were subject to heat shock that strongly induced expression of Hsps, particularly Hsp70. At time points thereafter, larvae were harvested and a macropatch electrode was utilized to record synaptic activity at individual, visualized boutons as the temperature of the preparation was raised in a stepwise fashion (Karunanithi et al., 1999). Increasing the temperature of the preparation resulted in failure of synaptic transmission, however, prior heat shock of the larvae sustained synaptic performance at high test temperatures through both pre- and postsynaptic alterations. Beneficial presynaptic modifications resulting from the larval 'preconditioning' were apparent since nerve impulses released more quantal units at high temperature and exhibited fewer failures of neurotransmitter release. In addition, beneficial postsynaptic modifications were reflected by the constant amplitude of quantal currents. The time course of these protective modifications of synaptic physiology paralleled the time course of Hsp70 induction. The protective effects of the prior heat shock on synaptic physiology was maximal at peak Hsp70 levels and declined as stress-induced Hsp70 decayed. These observations demonstrate that stress-induced neuroprotective mechanisms are operative that maintain the functionality of synapses by modifying their physiological properties.

Prior heat shock conferred protection to Drosophila synapses during subsequent thermal stress by stabilizing quantal size and reducing the decline of quantal emission at individual synaptic boutons. Expression of Hsp70 was not detectable in nonheat-shocked larvae, however, following heat shock 'preconditioning', it became the most prominent induced protein. To investigate whether Hsp70 is an important component of the synaptic protective response, transgenic Drosophila were engineered to contain 12 extra copies of the Hsp70 gene. Elevation of temperature induced a greater amount of Hsp70 in the transgenic larvae compared to an excision line that shared the same chromosomal sites of transgene insertion and flanking sequences but lacked the extra copies of the Hsp70 gene. This selective overexpression of Hsp70 enhanced the level of synaptic performance as assayed by measuring quantal content and percentage of success of synaptic transmission (Karunanithi et al., 2002). Use of a Drosophila mutant that fails to accumulate inducible Hsp70 revealed the compensatory upregulation of constitutively expressed Hsps and the preservation of synaptic thermoprotection (Neal et al., 2006). Heat shock 'preconditioning' has been shown to protect synaptic transmission in slice preparations from the mammalian brain (Kelty et al., 2002). This study also demonstrated that addition of exogenous Hsp70 to the medium of the brain slices protected synaptic transmission from thermal stress. These studies demonstrate that the protection conferred to the nervous system by heat shock 'preconditioning' has been extended to the level of synaptic function. Targeting Hsp70 to motor neurons

induced structural plasticity of axonal terminals that resulted in increased transmitter release at neuromuscular junctions at high temperature (Xiao et al., 2007). This protected larval locomotor activity from hyperthermia in Drosophila.

ASSOCIATION OF HEAT SHOCK PROTEINS WITH THE SYNAPSE

Hsps are composed of constitutively expressed members that are present in unstressed cells and inducible members that are expressed in response to stressful stimuli. Biochemical fractionation of tissue from the mammalian brain indicates that constitutively expressed Hsp90, Hsp60 and Hsc70 are associated with synaptic elements in the unstressed animal, suggesting that they play roles in normal synaptic function (Bechtold et al., 2000). Constitutive members of the Hsp70 multigene family are involved in the regulated release of neurotransmitters at synapses that depends on repeated cycles of exocytosis and endocytosis of synaptic vesicles within the presynaptic element (Bronk et al., 2001; Zinsmaier and Bronk, 2001). Together with cysteine-string protein, Hsc70 is a component of synaptic vesicle fusing complexes that form a synaptic chaperone machine (Chamberlain and Burgoyne, 2000; Bronk et al., 2001, 2005; Tobaben et al., 2001; Zinsmaier and Bronk, 2001; Dawson-Scully et al., 2007). Hsc70 is also associated with postsynaptic elements such as the postsynaptic density (Bechtold et al., 2000). DNAJ-like proteins, cysteine-string protein and Hsp40 have been identified at the synapse (Kohan et al., 1995; Suzuki et al., 1999; Ohtsuka and Suzuki, 2000). Following a physiological relevant increase in body temperature, Hsp70 is induced and is found associated with biochemically isolated synaptic elements including the postsynaptic density (Bechtold et al., 2000). Immunoelectron microscopy has provided visual confirmation of the localization of stress-inducible Hsp70, and also constitutively expressed Hsc70, to synaptic elements (Bechtold et al., 2000). Subcellular fractionation and immunoelectron microscopy have also demonstrated that hyperthermia-induced Hsp27 and Hsp32 are present in synaptic elements (Bechtold and Brown, 2000). Early induction of Hsp70 by chronic hypoxia stress has been shown to be critical for maintaining expression levels of presynaptic proteins (Fei et al., 2007). This study reports a direct interaction between Hsp70 and the presynaptic protein syntaxin. The positioning of Hsps at the synapse could facilitate the repair of stress-induced damage to synaptic proteins and contribute to neuroprotective mechanisms at the synapse.

LIPID RAFTS AND HEAT SHOCK PROTEINS

Lipid rafts are specialized plasma membrane microdomains that are enriched in cholesterol and sphingolipids that serve as major assembly and sorting platforms for signal transduction complexes (Brown and London, 1998; Simons and Toomre, 2000). The brain is enriched in lipid rafts. More than 1% of total brain protein is recovered in a lipid raft fraction, whereas less than 0.1% of total protein is associated with lipid rafts isolated from non-neural tissue (Maekawa et al., 2003). It

has been proposed that lipid rafts are important components of synapses critical for the maintenance of synaptic stability (Suzuki, 2002; Hering et al., 2003). A wide range of neurotransmitter receptors and constitutively expressed Hsp90, Hsc70, Hsp60 and Hsp40 are present in lipid rafts isolated from regions of the rat brain (Chen et al., 2005). Within 1 hr of hyperthermia, stress-induced Hsp70 was detected in lipid rafts isolated from the cerebellum (Chen et al., 2005). These observations indicate that constitutively expressed Hsps participate in the normal functioning of lipid rafts during neurotransmission events at synapses. After hyperthermia, the presence of stress-inducible Hsp70 in lipid rafts suggests a role in preserving the functional stability of lipid rafts and their associated signal transduction complexes at synapses.

STRESS-INDUCED ENHANCEMENT OF CONSTITUTIVELY EXPRESSED HSC70 IN SYNAPSE-RICH AREAS OF THE BRAIN

Hsp70 is a multi-gene family composed of stress-inducible Hsp70 and other members that are constitutively expressed (Hsc70). It is noteworthy that Hsp70 and Hsc70 exhibit similar molecular structures and biochemical features (Hightower et al., 1994). Heat shock 'preconditioning' triggers the synthesis of stress-inducible Hsp70, hence protective mechanisms have tended to focus on this stress-inducible Hsp. However, Hsc70 has also been hypothesized to play a role in thermotolerance and stress resistance (diIorio et al., 1996). In vitro studies on purified mammalian Hsc70 have reported that the protein undergoes a conformational change that activates its peptide/unfolded-protein binding activity as temperature is increased (Leung et al., 1996). This heat-induced Hsc70 conformational change may be associated with acquired thermotolerance (Leung et al., 1996).

Our studies have noted that constitutively expressed Hsc70 protein is enriched in the mammalian nervous system compared to non-neural tissue and is present at high levels in neuronal cell bodies (Manzerra et al., 1993, 1997; Manzerra and Brown, 1996). After thermal stress, overall neural levels of Hsc70, as determined by Western blotting, do not change. However, confocal immunocytochemistry detected an enhancement of Hsc70 in synapse-rich areas of the cerebral cortex that were identified by the synaptic marker synaptophysin (Chen and Brown, 2007a). The functioning of Hsc70 protein requires the assistance of Hsp40 that delivers protein candidates to Hsc70 and stimulates the ATPase actitivity of Hsc70 to mediate correct protein folding (Fan et al., 2003). The association of Hsc70 with Hsp40 was investigated by co-immunoprecipitation. This showed that Hsc70 and Hsp40 were associated in the unstressed brain, as expected, in Hsc70/Hsp40 chaperoning machinery engaged in the folding of newly synthesized proteins (Chen and Brown, 2007a). Thermal stress can induce protein unfolding (Lepock, 2005). Interestingly co-immunoprecipitation demonstrated that the association of Hsc70 and Hsp40 increased in the brain after heat shock (Chen and Brown, 2007a). Confocal immunocytochemistry revealed an increased co-localization of Hsc70 and Hsp40 in synapse-rich areas after heat shock. This could reflect the association of Hsc70

and Hsp40 in a synaptic chaperone machine that refolds synaptic proteins that have been perturbed by stress (Chen and Brown, 2007a).

These results could be interpreted to suggest that the heat shock response in the nervous system involves not only the induction of stress-inducible Hsps but also the translocation of constitutively expressed Hsc70 to synapse-rich areas where it participates with Hsp40 in neuroprotective mechanisms that preserve synaptic function during times of stress. An alternative possibility to explain the stress-induced enhancement of Hsc70 in synapse-rich areas is 'local translation' of Hsc70 mRNA at the synapse (see subsequent section entitled 'mRNA transport into post-synaptic dendrites of neurons'). A protective role for Hsc70 may be particularly relevant to differentiated neurons that characteristically exhibit high levels of Hsc70 and do not synthesize stress-inducible Hsp70 after thermal stress (Manzerra et al., 1993; Foster et al., 1995; Marcuccilli et al., 1996; Batulan et al., 2003). In contrast to neurons, glial cells demonstrate a strong induction of Hsps in response to hyperthermia in both tissue culture systems and in the intact nervous system (Brown, 1994; Foster et al. 1995; Foster and Brown, 1997; Brown and Sharp, 1999; Franklin et al., 2005). More severe stress, such as ischemia, results in induction of Hsp70 in neurons (Franklin et al., 2005).

TRANSFER OF HEAT SHOCK PROTEINS BETWEEN CELL TYPES IN THE NERVOUS SYSTEM

Transfer of Hsps between cell types in the nervous system was suggested by early work for the Tytell laboratory (Tytell et al., 1986). Heat shock induces the synthesis of Hsp70 in glial cells located in the sheath surrounding the squid giant axon and the rapid transport of this glial stress protein to the adjacent axonal process. This 'glial to neuron' transfer may provide a mechanism for fast delivery of neuroprotective Hsps to cellular processes distant from the neuronal cell body. Subsequent work has indicated that application of exogenous Hsps at neural injury sites is an effective strategy to maintain neuronal viability (Tytell et al., 1993; Houenou et al., 1996; Guzhova et al., 2001; Yu et al., 2001; Tidwell et al., 2004; Robinson et al., 2005; Tytell, 2005). Interestingly, hyperthermia results in the appearance of stress-inducible Hsp27 and Hsp32 in perisynaptic glial processes that surround and nurture synapses (Bechtold and Brown, 2000). These Hsps are subsequently transported to synaptic compartments. For additional discussion on extracellular release of Hsps in the nervous system and their effects on other neural cells, see Chapter 13 by Tytell et al.

mRNA TRANSPORT INTO POSTSYNAPTIC DENDRITES OF NEURONS

Using the electron microscope, Steward and Levy (1982) detected polyribosomes in the distal dendritic processes of neurons in the dendate gyrus. This raised the possibility that mRNAs could be transported to synapses and locally translated in

response to synaptic stimulation. Subsequent studies demonstrated that synapse-rich biochemical fractions could corporate labeled amino acids into protein (Rao and Steward, 1991; Weiler and Greenough, 1991; Torre and Steward, 1992). A functional role for dendritic protein synthesis was suggested by the observation that local protein synthesis in dendrites was required for the rapid enhancement of synaptic transmission by exposure to growth factor (Kang and Schuman, 1996). Current evidence suggests that dendritic protein synthesis contributes to various aspects of memory processing and synaptic remodeling and plasticity (Sutton and Schuman, 2006). It is now accepted that mRNAs localize to postsynaptic dendrites and that translation of these mRNAs is regulated in response to neuronal activity (Martin and Zukin, 2006; Schuman et al., 2006). Central questions that are being investigated include (1) what mRNAs are present in dendrites (2) how are they transported from the neuronal nucleus to postsynaptic dendrites (3) how is translation of these mRNAs regulated by synaptic activity and (4) what is the function of local translation at the synapse of these mRNAs (Martin and Zukin, 2006; Hirokawa, 2006; Schuman et al., 2006; Pfeiffer and Huber, 2006). Dendritic mRNAs have been found to encode a variety of proteins that extend beyond those involved in modulating synaptic plasticity. For example, synapse-associated mRNAs have been found to encode receptors, channels, signaling molecules, cytoskeleton proteins, adhesion molecules and factors involved in vesicle trafficking, protein synthesis and protein degradation (Moccia et al., 2003; Sung et al., 2004; Zhong et al., 2006).

Are mRNAs encoding heat shock proteins transported into the dendritic processes of neurons? Examination of the intracellular localization of mRNA encoding constitutively expressed Hsc70 protein demonstrated that it is localized to the cytoplasm of neuronal cells bodies in unstressed animals (Foster and Brown, 1996). Following a physiological relevant increase in body temperature, transport of hsc70 mRNA into dendritic processes was apparent in a range of neuronal cell types. These neuronal cell types did not induce hsp70 mRNA after hyperthermia, however hsp70 mRNA was strongly induced in glial cells and transported into cellular processes of these glial cells (Foster and Brown, 1996). The localization of hsc70 mRNA and hsp70 mRNA in the cellular processes of neural cells could provide a mechanism for local control of the synthesis of Hsps in cellular compartments, including the synapse, that are remote from the cell body.

HEAT SHOCK PROTEINS AND PROTEIN MISFOLDING

Neurodegenerative disorders, such as Alzheimer's disease, Parkinson's disease, and amyotrophic lateral sclerosis (ALS) have been termed 'protein misfolding disorders' (Selkoe, 2004; Muchowski and Wacker, 2005; Brown, 2007; Haass and Selkoe, 2007). They are characterized by accumulation of intracellular and extracellular protein aggregates, disruption of synaptic function and selective neuronal loss in the nervous system. These neurodegenerative diseases differ widely in frequency and impact different classes of neurons, however, increasing evidence supports the view that they exhibit common molecular mechanisms associated with

protein misfolding and aggregation (Forman et al., 2004; Ross and Poirier, 2004; Selkoe, 2004; Muchowski and Wacker, 2005). Recent evidence suggests that soluble oligomers of misfolded proteins interfere with synaptic function in Alzheimer's disease and other neurodegenerative disorders (Haass and Selkoe, 2007).

Manipulation of the cellular stress response involving induction of Hsps offers a therapeutic strategy to counter conformational changes in neural proteins that trigger cascades resulting in neurodegenerative diseases (Sherman and Goldberg, 2001; Meriin and Sherman, 2005; Muchowski and Wacker, 2005; Westerheide and Morimoto, 2005; Brown, 2007). Hsps are protein repair agents that provide a line of defense against misfolded aggregation-prone proteins (Muchowski and Wacker, 2005). Animal models of neurodegenerative diseases have demonstrated the beneficial effects of upregulation of Hsps on disease progression (Kieran et al., 2004; Kalmar et al., 2005; Muchowski and Wacker, 2005). Hsp70 overexpression has been reported to functionally protect synapses at the level of neurotransmission (Karunanithi et al., 2002) and synaptic function is disrupted in neurons during disease progression (Li et al., 2000, 2003; Lee et al., 2003; Smith et al., 2005a, b; Cummings et al., 2007; Haass and Selkoe, 2007). This has led to the quest for pharmacological agents that can induce the heat shock response in the nervous system as a therapeutic approach to counter neurodegenerative diseases. Upregulation of a combination of Hsps, rather than a single Hsp, will likely yield added benefits (Jana et al., 2000; Patel et al., 2005). It has been suggested that the finely balanced, concerted action of a set of Hsps could best be achieved by the development of agents that target activation of heat shock transcription factor 1 (HSF1), the master regulator of stress-inducible genes (Westerheide and Morimoto, 2005; Zourlidou et al., 2007).

A collaborative drug screen aimed at identifying candidates from a panel of existing drugs has identified celastrol as a potential neuroprotective agent (Abbott, 2002; Heemskerk et al., 2002). The screen utilized tissue culture assays to score drugs on their ability to suppress aspects associated with neurodegenerative diseases such as protein aggregation. Celastrol has been used in traditional Chinese medicine to treat various ailments such as inflammation (Pinna et al., 2004) and rheumatoid arthritis (Tao et al., 2002). This compound exhibits neuroprotective properties in animal models of neurodegenerative diseases such as Parkinson's disease (Cleren et al., 2005), ALS, (Kiaei et al., 2005), and Huntington's disease (Cleren et al., 2005; Wang et al., 2005). Celastrol has been shown to activate heat shock transcriptional factor HSF1 in undifferentiated neuroblastoma cells (Westerheide et al., 2004).

Recent studies have demonstrated that celastrol induces a set of Hsps in differentiated neurons grown in tissue culture (Chow and Brown, 2007). Our current work indicates that these celastrol-induced Hsps are transported down neuronal processes towards synaptic termini. The differentiation status of neurons is of particular importance because differentiated neurons in both in vivo and in vitro systems have been reported to be refractory to Hsp induction following conventional heat shock (Manzerra et al., 1993; Foster et al., 1995; Dwyer et al., 1996; Marcuccilli

et al., 1996; Hatayama et al., 1997; Batulan et al., 2003). The celastrol experiments were carried out on human and rodent differentiated neurons in order to explore species-specific differences in Hsp induction patterns that might influence the translation of observations on animal-based models of neurodegenerative diseases to the actual human condition. This led to the finding that celastrol induced a wider set of potentially neuroprotective Hsps, including Hsp70B', in differentiated human neurons compared to rodent neurons (Chow and Brown, 2007).

The human genome includes members of the Hsp70 multigene family, such as Hsp70B', that are not present in the genomes of rodents (Parsian et al., 2000; Noonan et al., 2007). The Hsp70B' gene arose after the divergence of rodents and humans and hence is not present in animal models of neurodegenerative diseases as a potential beneficial factor to combat misfolded aggregation prone-proteins. The Hsp70B' protein and stress-inducible Hsp70 share 84% sequence identify, however, differences in the substrate binding pocket and activation profiles may confer Hsp70B' with a distinct cellular role (Noonan et al., 2007). Hsp70B' has not been studied in the field of human neurodegenerative diseases. We are presently investigating the binding partners and potential neuroprotective properties of Hsp70B', in addition to determining whether it localizes to synaptic termini and protects synapses from stressful stimuli. Celastrol is a promising candidate as a therapeutic agent to counter neurodegenerative diseases with the attractive feature of upregulating a wider set of Hsps, including Hsp70B', in differentiated human neurons compared to rodent neurons (Chow and Brown, 2007).

NEURONAL EXPRESSION OF CONSTITUTIVE HEAT SHOCK PROTEINS AND FREQUENCY OF NEURODEGENERATIVE DISEASES

Constitutively expressed Hsc70 is enriched in the mammalian nervous system relative to non-neural tissue and is present at high levels in neuronal cell bodies (Manzerra et al., 1993, 1997; Manzerra and Brown, 1996). Following thermal stress, Hsc70 is enhanced in synapse-rich areas of the brain where it could play roles in synaptic protective mechanisms (Chen and Brown, 2007a). Levels of Hsc70 have been compared in different classes of neurons that are affected in different neurodegenerative diseases (Chen and Brown, 2007b). Motor neurons in the spinal cord impacted in a low frequency disease such as ALS, demonstrated very high levels of Hsc70, whereas neurons in the hippocampus and entorhinal cortex affected in a high frequency disease such as Alzheimer's, showed comparatively low levels of Hsc70. Intermediate levels of Hsc70 were apparent in neurons of the substantia nigra that are impacted in an intermediate frequency disease such as Parkinson's disease. The differing levels of constitutively expressed Hsc70 in different neuronal populations may confer a variable buffering capacity against protein misfolding disorders that correlates with the relative frequency of these diseases in the human population (Chen and Brown, 2007b). Variable levels of Hsc70 could be present at synaptic termini in the different neuronal cell types, resulting in variability in the degree of

potential synaptic protection. Neurons may rely on their constitutive levels of Hsc70 as a 'pre-protection' mechanism for defense against aggregation-prone misfolded proteins that accumulate following stressful stimuli or during of the progression of neurodegenerative diseases.

CONCLUDING REMARKS

Synapses are critical sites of information transfer in the nervous system and their functionality must be preserved under stressful conditions to prevent communication breakdown. Heat shock proteins localize to components of the synapse and play roles in neurotransmission events including the functional protection of synapses against stressful stimuli. Synaptic dysfunction is a feature of neurodegenerative disorders and manipulation of the heat shock response is a potential strategy to mitigate disruption of synaptic function that occurs during disease progression.

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