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Heat shock protein defenses in the neocortex and allocortex of the telencephalon

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ABSTRACT

The telencephalic allocortex develops protein inclusions before the neocortex in many age-related proteinopathies. One major defense mechanism against proteinopathic stress is the heat shock protein (Hsp) network. We therefore contrasted Hsp defenses in stressed primary neocortical and allocortical cells. Neocortical neurons were more resistant to the proteasome inhibitor MG132 than neurons from 3 allocortical subregions: entorhinal cortex, piriform cortex, and hippocampus. However, allocortical neurons exhibited higher MG132-induced increases in Hsp70 and heat shock cognate 70 (Hsc70). MG132-treated allocortical neurons also exhibited greater levels of protein ubiquitination. Inhibition of Hsp70/Hsc70 activity synergistically exacerbated MG132 toxicity in allocortical neurons more than neocortical neurons, suggesting that the allocortex is more reliant on these Hsp defenses. In contrast, astrocytes harvested from the neocortex or allocortex did not differ in their response to Hsp70/Hsc70 inhibition. Consistent with the idea that chaperones are maximally engaged in allocortical neurons, an increase in Hsp70/Hsc70 activity was protective only in neocortical neurons. Finally, the levels of select Hsps were altered in the neocortex and allocortex in vivo with aging.

1. Introduction

The modern neocortex or isocortex of the telencephalon can readily be distinguished at the structural and functional levels from the phylogenetically primitive allocortex (comprised of the paleocortex and archicortex). In Parkinson's disease (PD) and Alzheimer's disease (AD), the neocortex is affected by α-synuclein and tau inclusions after the allocortex (Braak et al., 1993, 2003a, 2003b, 2006; Duyckaerts et al., 2009). The reason underlying this striking difference is still unknown. One explanation may be that protein-misfolding stress (proteotoxicity) travels through circuits in a staggered fashion, so that regions are sequentially collared into bearing inclusions depending on their individual projection patterns. A second possibility is that brain regions exhibit differences in their vulnerability to proteotoxicity and can thus keep inclusions at bay for varying periods. We propose that these 2 scenarios occur simultaneously and have examined the second possibility here.

The concept that pathology is transmitted from cell to cell has gained considerable traction in recent years (Desplats et al., 2009; Hansen et al., 2011; Luk et al., 2012; Volpicelli-Daley et al., 2011). However, transmissibility cannot fully explain the topography of inclusions in PD and AD because not all the areas connected to an affected region develop protein inclusions and die. For this reason, it has been hypothesized that inherent differences in vulnerability also help determine cell fate (Braak et al., 2006; Dickson, 2007). It is important to identify the natural defenses against proteotoxic stress in resilient brain regions because they may explain why neurodegenerative diseases usually do not appear until old age and are slow to progress.

The major form of stress in neurodegenerative disorders is thought to be proteotoxic stress, and one of the main lines of defense against these conditions is the heat shock protein (Hsp) family of chaperones, part of the essential vitagene network (Cornelius et al., 2013). For example, Hsp70 refolds denatured proteins and guides irreparably damaged proteins to the

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proteasome or lysosome for degradation (Aridon et al., 2011; Kalia et al., 2010; Lanneau et al., 2010). Hsp70 also offsets apoptosis. Hsp70 ameliorates 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine, α-synuclein, and β-amyloid toxicity (Dong et al., 2005; Klucken et al., 2004; Magrane et al., 2004; Moloney et al., 2014; Nagel et al., 2008). However, the impact of Hsp70 on regionally selective vulnerability to proteotoxicity is poorly understood.

We recently gathered evidence that the neurons of the neocortex and allocortex have different intrinsic vulnerabilities to oxidative and proteotoxic stress (Posimo et al., 2013). Primary neocortical cultures were more resistant to proteasome inhibitors such as MG132 but were more vulnerable to hydrogen peroxide. It is possible that the increased susceptibility of the allocortex to proteotoxicity may partly underlie the spatiotemporal pattern of cortical inclusions in PD and AD. Relative to the allocortex, neocortex cultures also exhibited lower levels of ubiquitinated proteins and less loss of proteasome activity in response to MG132. However, allocortex cultures exhibited greater increases in Hsp70 on MG132 treatment. To determine the functional consequences of these regional differences in Hsps, we first reestablished the neocortical and allocortical culture model with higher neuronal purity, improved basal survival, and more robust regional differences in MG132 vulnerability than previously reported (Posimo et al., 2013). Second, we compared the vulnerability of the neocortex to that of 3 allocortical subregions: entorhinal cortex, piriform cortex, and hippocampus. The piriform cortex is comprised of 3 layers, meeting the classic definition of the trilaminar allocortex. The entorhinal cortex is a transitional zone and composed of >3 layers, but it is closely associated with the allocortex and classified as periarchicortex (Braak, 1980; Creutzfeldt, 1995; Filimonoff, 1947; Peters and Jones, 1984). Hippocampus is also considered part of the archicortex. All 3 regions are more susceptible to tau inclusions than the neocortex (Braak and Braak, 1995; Braak et al., 1993, 2006; Duyckaerts et al., 2009). Third, we examined the effects of MG132 and the oxidative toxin paraquat on Hsps in primary cortical cultures and the impact of Hsp70 and heat shock cognate 70 (Hsc70) inhibition and activation on neocortical and/or allocortical vulnerability to these compounds. Finally, we measured the impact of aging on Hsp expression in the primary sensorimotor neocortex and entorhinal allocortex in vivo, as aging is a major risk factor for neurodegeneration and can be leveraged as a natural model of proteotoxic and oxidative stress (Keller et al., 2000b).

2. Methods

2.1. Animals

Animal use was approved by the Duquesne University Institutional Animal Care and Use Committee and carried out in accordance with the principles outlined in the National Institutes of Health Guide for the Care and Use of Laboratory Animals. Female Sprague Dawley rats were fed ad libitum and singly housed in a room maintained at a constant temperature with a 12-hour light-dark cycle. Rats were sacrificed at 2–3.9 (n = 6), 4–6 (n = 6), 8–9 (n = 6), 16–18.9 (n = 5), and 19–22 (n = 6) months of age for the aging study. These female rats formed part of an in-house breeding colony designed to generate rat pup tissue for postnatal cultures (see Section 2.3). The tissue was dissected according to the definitions in the Paxinos rat atlas (Paxinos and Watson, 1998).

2.2. Antibodies and chemicals

Primary and secondary antibodies are listed in Supplementary Tables 1 and 2. The proteasome inhibitor MG132 was purchased from EMD Millipore (Billerica, MA, USA, Cat. no. 474790). Hsp70 and Hsc70 activity was inhibited with the previously characterized compounds VER155008 (R&D Systems, Minneapolis, MN, USA; Chatterjee et al., 2013; Massey et al., 2010; Saykally et al., 2012; Schlecht et al., 2013) and MAL3-101 (Adam et al., 2014; Braunstein et al., 2011; Hatic et al., 2012; Huryn et al., 2011; Kilpatrick et al., 2013). Hsp70 activity was enhanced with 115-7c (MAL1-271) (Kilpatrick et al., 2013; Wisen et al., 2010). Heme oxygenase-1 (HO1) was inhibited with tin protoporphyrin (SnPP) (Drummond and Kappas, 1981). All toxins and inhibitors were stored at −80 °C as a 10-mM stock solution in dimethyl sulfoxide, except for the oxidative toxin paraquat (Sigma-Aldrich, St. Louis, MO, USA), which was dissolved in phosphate-buffered saline (PBS; 100 mM).

2.3. Primary cultures

Neocortical tissue and tissue from the entorhinal allocortex, piriform allocortex, and hippocampal allocortex were dissected from the brains of postnatal day 1 or 2 Sprague Dawley rats (Charles River, Wilmington, MA, USA). Cells were dissociated and plated as previously described (Posimo et al., 2013), with the exception that cultures were incubated in Neurobasal-A media (Gibco; Life Technologies) supplemented with 2% vol/vol serum-free B-27 (Gibco; Life Technologies) and 2-mM L-glutamine. Cultures were treated with an equal vol/vol of vehicle or toxins on day in vitro 2 (DIV2). On DIV3, a full media exchange was performed. On DIV4, cell viability assays were performed as described in the following section. In our experience, cells treated with proteasome inhibitors do not respond with much cell loss until 2 days after treatment. Thus, a 48-hour time point was used for the viability assays.

Primary astrocytes were also harvested from the neocortex and allocortex. For the astrocyte cultures, the entorhinal cortex was combined with the piriform cortex to generate sufficient astrocytic material. Briefly, the tissue was dissociated in Dulbecco's Modified Eagle Medium (Gibco; Life Technologies) supplemented with 10% FetalClone III (HyClone, Thermo Scientific) and 1% penicillin and streptomycin (Gibco; Life Technologies) after incubation with 0.25% trypsin with EDTA (Invitrogen; Life Technologies). After 7–9 days, cultures were placed overnight on an orbital shaker at 260 rpm. Two to 3 days later, astrocytes were passaged and seeded onto plates. Astrocyte cultures were treated with toxins on DIV5 and assayed on DIV7.

2.4. Viability assays

Cell viability in neuronal cultures was assessed by quantifying adenosine triphosphate (ATP) levels using the CellTiter-Glo assay (Promega, Madison, WI, USA) and by quantifying levels of the neuronal marker microtubule-associated protein 2 (MAP2) using an In-Cell Western assay, as described (Posimo et al., 2013, 2014). The infrared In-Cell Western assay for MAP2 was imaged on an Odyssey Imager and analyzed with Odyssey software (version 3.0, LI-COR). Astrocyte cultures were stained with Hoechst (10 μg/mL Hoechst 33258, bisBenzimide) in PBS with 0.3% Triton-X for 15 minutes for blinded cell counts. Astrocytes were also stained with the infrared nuclear stain DRAQ5 (1:10,000, 700 nm; BioStatus, Shepshed, Leicestershire, UK) and assayed on the Odyssey Imager to validate the Hoechst cell count data. In addition, cultures were immunocytochemically stained for MAP2, the astrocyte marker glial fibrillary acidic protein (GFAP), and/or the synaptic protein synaptophysin in the visible wavelengths as described (Posimo et al., 2013). $MAP2^+$ neurons, $GFAP^+$ astrocytes, and/or Hoechst-stained nuclei were then counted by a blinded observer at 200× magnification in a 0.213-mm^2 field of view (3 fields per well).