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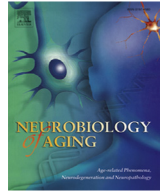

Age- and brain region-specific differences in mitochondrial bioenergetics in Brown Norway rats

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ABSTRACT

Mitochondria are central regulators of energy homeostasis and play a pivotal role in mechanisms of cellular senescence. The objective of the present study was to evaluate mitochondrial bioenergetic parameters in 5 brain regions (brain stem [BS], frontal cortex, cerebellum, striatum, hippocampus [HIP]) of 4 diverse age groups (1 month [young], 4 months [adult], 12 months [middle-aged], 24 months [old age]) to understand age-related differences in selected brain regions and their possible contribution to age-related chemical sensitivity. Mitochondrial bioenergetic parameters and enzyme activities were measured under identical conditions across multiple age groups and brain regions in Brown Norway rats ($n = 5$/group). The results indicate age- and brain region–specific patterns in mitochondrial functional endpoints. For example, an age-specific decline in ATP synthesis (State III respiration) was observed in BS and HIP. Similarly, the maximal respiratory capacities (State V_1 and V_2) showed age-specific declines in all brain regions examined (young > adult > middle-aged > old age). Amongst all regions, HIP had the greatest change in mitochondrial bioenergetics, showing declines in the 4, 12, and 24-months age groups. Activities of mitochondrial pyruvate dehydrogenase complex and electron transport chain complexes I, II, and IV enzymes were also age and brain region specific. In general, changes associated with age were more pronounced with enzyme activities declining as the animals aged (young > adult > middle-aged > old age). These age- and brain region-specific observations may aid in evaluating brain bioenergetic impact on the age-related susceptibility to environmental chemical stressors.

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1. Introduction

There are currently an estimated 6 million Americans at the age of 85 and older and the number is constantly growing. The World Health Organization estimates that the worldwide aged population (65+ years) will increase more than threefold by 2050 (World Health Organization, 2011). Factors that affect susceptibility to environmental chemical exposures at different life stages are an area of growing concern in the risk assessment of human health. Animal models used to investigate the role of aging on physiological endpoints have demonstrated age-specific responses to environmental chemical stressors (Elder et al., 2000; Kodavanti et al., 2011; MacPhail et al., 2012). Thus, environmental chemicals may contribute to susceptibility by affecting the aged differently from young adults (Park et al., 2005; Royland et al., 2012). Our previous studies demonstrate the complexity of the interaction between life stage and environmental chemical exposure, highlighting a shift in neurotoxicity and oxidative stress dose response as a result of age, different responses across brain regions, and showing senescent rats to be more sensitive (Kodavanti et al., 2011; MacPhail et al., 2012). Research to better understand the biological processes of aging, and to learn about chemically induced susceptibility, is needed to optimize risk assessment and to minimize health risks in senior populations.

The brain is a unique and multifunctional organ consisting of a heterogeneous composition of cell types (neurons and glial cells) with distinct structural regions (cortex, hippocampus [HIP], striatum [STR], cerebellum [CER], brainstem [BS], and so forth) and functional areas (motor vs. sensory vs. memory). Relative to other organs, the brain has low levels of stored glycogen, using circulatory glucose almost exclusively as its energy source under normal

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conditions. It is also highly metabolic, consuming about 20% of the energy in the body even though it constitutes only 2% of total body mass (Silver and Erecinska, 1998). The senescent brain is distinguished by structural changes, changes in metabolic activity, and a gradual decline in physiological function (Andrews-Hanna et al., 2007; Bishop et al., 2010). In fact, brain aging is associated with many neurodegenerative disorders such as Alzheimer's and Parkinson's diseases (Mattson et al., 2002).

Biological theories of aging have been put forward, correlating a decline in physiologic function with an accumulation of damage due to lifetime exposure to toxic substances. The mitochondrial theory of aging, based on the free radical theory of aging proposed by Harman in the 1950s (Harman, 1956), is one of the most prominent and accepted hypotheses (Alexeyev, 2009; Cadenas and Davies, 2000; Harman, 1994). It proposes that oxidative metabolism produces highly reactive radicals that subsequently damage cellular lipids, proteins, and nucleotides. The free radical theory of aging was further developed into the mitochondrial theory of aging, which proposes that over time, reactive oxygen species (ROS) produced during normal energy metabolism result in an accumulation of mutations to mitochondrial DNA (mtDNA), ultimately leading to loss of mitochondrial respiratory chain function and decreased ATP production in aging (Balaban et al., 2005; Kelly, 2011; Wallace, 2005; Weinert and Timiras, 2003).

The slowdown in age-related ATP production may have adverse consequences on other energy-dependent cellular processes (Duchen, 2004; Geddes and Sullivan, 2009; Kann and Kovacs, 2007; Yonutas et al., 2015). For example, as brain mitochondria age with a subsequent decline in oxidative phosphorylation, brain behavioral correlates also may be predicted to decline. In addition, the brain contains high levels of ROS–sensitive unsaturated fatty acids, a high-iron content with potential for redox reactions, and low-antioxidant defenses, thereby making it a prime target for oxidative stress (Floyd and Hensley, 2002). Different brain regions may respond differently to age-induced mitochondrial dysfunction as a result of varying oxidative phosphorylation and ATP demands and innate differences in capacities to combat oxidative stress and damage (Hatefi, 1985; Sullivan and Brown, 2005; Wallace, 2005; Yonutas et al., 2015). In a previous study of nonhuman primates (Pandya et al., 2015), an age-dependent decline in whole brain mitochondrial ATP production was found. Moreover, significant decreases in mitochondrial ATP synthesis, pyruvate dehydrogenase complex (PDHC) enzyme activity, and calcium buffering capacity parameters in the basal ganglia of aged monkeys significantly correlated with motor function deficits (Pandya et al., 2015).

In this study, we carried out an in-depth, comprehensive study to advance our understanding of the role of mitochondrial bioenergetic function in aging. The study was carried out in the Brown Norway rat, a model of healthy aging which is known to age without many of the traits that confound aging studies in other rat strains (e.g., cancer, fat accumulation, and so forth; Wolden-Hanson et al., 1999). Mitochondrial bioenergetics and enzyme activities were evaluated at multiple ages (1, 4, 12, and 24 months) in 5 different brain areas (BS, FC, CER, STR, and HIP). Briefly, mitochondrial respiration parameters, for example, State III (ATP synthesis capacity), State IV (proton leakage), and State V (maximal electron transport) were measured together with mitochondrial enzyme activities (PDHC and Complexes I, II, and IV). These parameters reflect overall bioenergetic efficiency of brain region mitochondria at various ages. To our knowledge, such a novel, systematic, and comprehensive aging study has not been conducted. The multidimensional design of the study will provide valuable insight into changes in individual brain region metabolic status in response to aging. These data on mitochondrial bioenergetics will serve as a basic platform for future evaluation of multiple chemical and nonchemical stressors that may differentially target energy metabolism with respect to brain region or animal life stage. In addition, information gained in this study will aid in understanding environmental chemical-induced sensitivity in subsets of the population, an important aspect of community health–related research.

2. Materials and methods

2.1. Animals

Male Brown Norway rats from 4 different life stages were examined (i.e., 1 month = young, 4 months = adult, 12 months = middle-aged, and 24 months = old age). N = 5 rats/group for 4-, 12-, and 24-month old animals or 10 rats/group for 1-month old animals (2 animals pooled/sample). Ages were chosen to mimic significant human life stages important to toxicity susceptibility evaluations, that is, juvenile, postpubertal young adults, mature adults, and aging adults. Animals were purchased from the National Institutes of Health (National Institute of Aging) animal facilities at an appropriate age, less 2 weeks for acclimation to the new facility and to allow for experimental preparations. All animal protocol and procedures were approved by the University of Kentucky Institutional Animal Care and Use Committee. Animals were maintained at University of Kentucky animal facilities on a regular 12 hours light/12 hours dark cycle in temperature-controlled rooms (25 °C), group housed at 2 rats per cage, and provided with ad lib food and water. To limit age variability within groups, all animals were used within 3 days for the 1 month age group, and to a maximum of 14 days for the 4-, 12-, and 24-month age groups. After the 1-week acclimatization, all animals were randomly assigned dates of sacrifice. Two animals per day (except for 1-month animals where 2 animals were pooled per single sample giving 4 animals/day) were sacrificed in the morning between 9 and 10 AM, and tissues processed for mitochondrial isolation and bioenergetics parameter measurements.

2.2. Chemicals

All chemicals for mitochondrial isolation and bioenergetics assessments were obtained from Sigma-Aldrich (St. Louis, MO, USA). Seahorse Extracellular Flux cartridges were purchased from Seahorse Bioscience (North Billerica, MA, USA).

2.3. Isolation of mitochondria

Mitochondria from all 5 brain regions from randomized 2 age groups/day were isolated together under identical experimental conditions using an established Ficoll-based mitochondrial ultrapurification procedure (Pandya et al., 2007, 2009, 2011; Sullivan et al., 2007). Throughout the mitochondrial isolation period, samples were kept at 4 °C, unless otherwise specified. Animals were asphyxiated with CO_2 and rapidly decapitated. After decapitation, the brain was rapidly removed and placed in a beaker of ice-cold (4 °C) mitochondrial isolation buffer (MIB) composed of 215 mM Mannitol, 75-mM Sucrose, 0.1 % bovine serum albumin (BSA), 1 mM EGTA, and 20 mM HEPES at pH 7.2. All anatomical regions of interest were rapidly dissected apart, placed in prelabeled 5-mL tubes containing 1 mL of MIB buffer, and homogenized. Tissue homogenates were centrifuged at 1300 × g for 3 minutes to remove cell debris and nuclei, and the collected supernatant was then centrifuged at 13,000 × g for 10 minutes to pellet the crude mitochondria. The resultant crude mitochondrial pellet was then resuspended in 500 μL of MIB and kept in a nitrogen cell decompression bomb (model 4639, Parr Instrument Co., Moline, IL, USA) at

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