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Gender- and region-dependent changes of redox biomarkers in the brain of successfully aging LOU/C rat.

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ABSTRACT

The Lou/C (LOU) rat is an obesity resistant strain with higher longevity and healthspan than common rats. The management of oxidative stress being important to successful aging, we characterized this process in the aging LOU rat. Male/female LOU rats were euthanized at 4, 20, 29 months. Macrodissected hippocampus, striatum, parietal cortex, cerebellum were assayed for tissue concentrations of glutathione (GSH), gamma-glutamyl-cysteine-synthetase (γ-GCS), total thiols, protein carbonyls, mRNAs of clusterin and the known protective enzymes thioredoxine-1 (TRX-1), glutaredoxine-1 (GLRX-1), superoxydismutase-1 (SOD-1). Brain levels of GSH, γ-GCS, total thiols remained constant with age, except for GSH and γ-GCS decreases in females. Clusterin, TRX-1, GLRX-1, SOD-1 mRNA levels were maintained or increased in the hippocampus with age. Age-dependency of the markers differed between sexes, with SOD-1 and TRX-1 decreases out of hippocampus in females. Since antioxidants were reported to decrease with age in the brain of Wistar rats, maintenance of GSH levels and of protective enzymes mRNA levels in the LOU rat brain could contribute to the preservation of cognitive functions in old age. Altogether, the successful aging of LOU rats may, at least in part, involve the conservation of functional antioxidant mechanisms in the brain, supporting the oxidative stress theory of aging.

1. Introduction

Aging involves complex biological changes controlled by genetic, epigenetic and environmental factors (Gems 2014). For example, aging is commonly associated with progressive decline in cognitive performances in humans and rodents (Glisky, 2007; Hedden and Gabrieli, 2004). Cognitive impairment can occur during physiological aging, *i.e.* in the absence of neurodegenerative diseases (Boyle et al., 2013). Considering the current increase of elderly populations, this phenomenon becomes a socioeconomic issue. The present lack of knowledge about underlying mechanisms hampers the development of interventional strategies to prevent such decline and requires the study of new animal models. In this respect, the inbred LOU/C (LOU) rat (Bazin et al., 1986) is of great interest. It has been identified as a model of resistance to the development of obesity as a function of age (Couturier et al., 2002), which possibly relies on hormonal (Kappeler et al., 2004; Veyrat-Durebex et al., 2005) and metabolic characteristics (Boghossian et al., 2002; Helies et al., 2005; Mitchell et al., 2006; Perrin et al., 2003; Soulage et al., 2008), including food intake (Alliot et al., 2002) and efficiency (Veyrat-Durebex et al., 2009). It is considered a model of healthy aging with a longer lifespan reaching up to 42 months of age (Ménard et al., 2014) and a lower morbidity than most laboratory rat strains (Alliot et al., 2002). In LOU but not Sprague–Dawley rats, spatial memory remains intact at least up to 24 months of age in comparison to 3-months-old rats (Kollen et al., 2008; Kollen et al., 2010), and recognition up to 42 months (Ménard et al., 2014). Such better memory capacity has been associated with significantly higher level of the neuroprotective and memory-enhancing brain-derived neurotrophic factor (BDNF) in the hippocampus of LOU rats as compared to age-matched Wistar rats (Silhol et al., 2008). Also, the ionotropic and metabotropic glutamate receptor levels were shown to be preserved in mature (12 months), old (24-

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months) and very old (38–42 months) LOU rats as compared to young (6-months) ones (Ménard et al., 2014). Beyond these studies, the regulation of OS markers remained to be investigated in the LOU rat brain.

It is postulated that free radical production and accumulation of oxidative damage are among the prime candidates responsible for deleterious effects of aging and age-associated neurodegenerative disorders (Harman, 1972; Liochev, 2013). In contrast, factors increasing resistance to OS should have anti-aging benefits and may lead to increased life span. Many endogenous protective molecules are upregulated by free radicals, suggesting that these toxic compounds could trigger adaptations to counteract oxidative stressors (Holmström and Finkel, 2014). Considering the long life expectancy of the LOU rat, with maintenance of several physiological functions in old age including spatial and recognition memory (Ménard et al., 2014), we hypothesize that its successful aging might be related to a low production of reactive oxygen species (ROS) and/or high resistance against OS in the brain. Levels of antioxidants and antioxidant enzymes are closely related to cellular responses to various stressors. Among them, the glutathione (GSH) pathway, which includes glutathione peroxidase (GSH-PX), glutathione reductase (GRX), glutathione transferase (GST), thioredoxin (TRX) and glutaredoxin (GLRX), plays a crucial role in cellular response against ROS and peroxides, particularly in the maintenance of protein sulfhydryl groups. Moreover, these antioxidants are key effectors of the redox homeostasis which regulates various intracellular functions including stress response and cell growth (Holmström and Finkel, 2014). For example, TRX and GLRX are important in antioxidant defense and their expression is regulated through transcription factors such as NF-κB (Sakurai et al., 2004). Finally, it has been shown that these antioxidants are involved in the extension of life span (Mitsui et al., 2002).

Despite its physiological interest as a model of healthy aging, no study has examined the antioxidant homeostasis in the brain of the LOU rat. The aim of the present study was therefore to investigate whether or not successful aging of male and female LOU rats involves maintenance of antioxidant capacities in brain regions associated with diverse human neurodegenerative disorders like amyotrophic lateral sclerosis, Alzheimer's or Parkinson's diseases, (*e.g.* hippocampus, striatum, parietal cortex) in comparison with cerebellum as a degeneration-resistant structure (Thal et al., 2004). We have measured levels of glutathione (GSH), γ-glutamylcysteine synthetase (γ-GCS), total thiols, carbonylated proteins, mRNAs levels of thioredoxin-1 (TRX-1), glutaredoxin-1 (GLRX-1), superoxide-dismutase-1 (SOD1), clusterin/apolipoprotein J (CLU), and endogenous antioxidant capacity in rats of both sexes at three different ages. Markers of oxidative damage were not drastically altered in LOU rat brain during aging, and antioxidant effectors were either maintained or increased, in a sex- and region-specific manner.

2. Experimantal procedure

2.1. Animals and diets

4-, 20- and 29-month-old male and female LOU rats were obtained from the Aging LOU Rat Colony Infrastructure of the Quebec Network for Research on Aging (www.rqrv.com/fr/infra_colonie.php). The 4-month-old females were virgins. Their ancestors were obtained at three months of age (J Alliot, Université Blaise Pascal, Clermont-Ferrand, FR) and breeding has been performed in CRCHUM animal facilities since then. The LOU rats were fed the SAFE R03 growing diet for three weeks after weaning and the SAFE R04 maintenance diet thereafter (Perotech Sciences Inc., Toronto, ON, Canada) as previously described (Duque et al., 2009; Veyrat-Durebex et al., 2005) and had free access to chow and water. They were housed in standard plastic cages (2–3/cage) in temperature (22 °C), humidity (65%) and lighting-controlled (12:12-h light-dark cycles; light on at 07:00) rooms. Longevity characteristics of these LOU rats were comparable to those previously reported (Alliot et al., 2002). Average body weights at sacrifice of the cohorts were as follows: 260 ± 5 g for 4-month-old males (n = 11), 166 ± 3 g for 4-month-old females (n = 14), 378 ± 10 g for 20-month-old males (n = 13), 184 ± 3 g for 20-month-old females (n = 14), 360 ± 15 g for 29-month-old males (n = 10), and 203 ± 8 for 29-month-old females (n = 10).

All rats were euthanized by rapid decapitation, in a block-design fashion, between 08:30 and 13:00 h, after overnight food deprivation. Brains were rapidly macrodissected and hippocampus, striatum, parietal cortex and cerebellum were flash-frozen in liquid nitrogen and stored at −80 °C. Post-mortem macroscopic evaluation revealed no gross pathology or tumors in old animals. The animal protocols were approved by the Animal Care Committee of the CRCHUM in compliance with the Canadian Council for Animal Care guidelines.

2.2. Tissue protein and RNA extraction

In a first series of experiments, frozen tissues from 6 rats per age group (4, 20 and 29 months) and sex were homogenized with a glass/teflon potter tissue grinder in 20 volumes of phosphate buffer containing 1 mM ethylene-diamine-tetra-acetic acid (EDTA) and 1:100 protease inhibitor cocktail (Sigma–Aldrich Canada, Oakville, ON; P8340). Homogenates were centrifuged (10 min, 1000 × *g*, 4 °C) and supernatants removed and stored at −80 °C. Proteins contents were quantified using the BCA protein assay kit (Pierce Biotechnology, Rockford, IL, USA).

In a second series of experiments, total RNA was extracted from frozen tissues of alternate rats including 4-month-old males (n = 4), 4-month-old females (n = 5), 20-month-old males (n = 5), 20-month-old females (n = 5), 29-month-old males (n = 5), 29-month-old males (n = 5). Each sample was first homogenized in 400 μL TRIzol (Invitrogen Canada Inc., Burlington, ON). Then chloroform-solubilized, isopropanol-precipitated RNA was resuspended in 10 μL RNAse-free distilled water (Ambion, Streetsville, ON, Canada). RNA concentration and purity were assessed by spectrometry: 260/280 nm ratios were all above 1.9 and 20–100 μg total RNA per brain region was obtained. RNA extracts were frozen at −80 °C. Quality control of RNA samples was assessed using a 2100 Bioanalyzer and RNA 6000 Nano LabChip kit (Agilent Technologies, Mississauga, ON, Canada). The RNA integrity number of all samples was >9.0.

2.3. Reduced glutathione assay

Reduced glutathione (GSH) was measured using monochlorobimane (MCB) which forms a fluorescent adduct with GSH (Kamencic et al., 2000). MCB was added at 100 μM per well to 96-well plates containing 50 μg of proteins from brain homogenates, and the reaction was started with 1 U/mL GST. After 30 min of incubation at room temperature (RT), the fluorescence was recorded with λex at 360 nm and λem at 460 nm using a Synergy HT multi-detection microplate reader from BioTek (Winooski, VT, USA). Experimental values were quantified by comparison to a standard curve performed with reduced GSH. The intra-coefficient of variation (CV) assay was ≤9 % in all experiments and the inter-assay CV was ≤3%.

2.4. Western blotting of γ-GCS and carbonylated proteins

For γ-GCS quantification, brain homogenates (30 or 50 μg proteins) were separated on 10% or 12% SDS-PAGE gels and transferred

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