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Redox changes in the brains of reproductive female rats during aging

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ABSTRACT

Reproduction is a critical and demanding phase of an animal's life. In mammals, females usually invest much more in parental care than males, and lactation is the most energetically demanding period of a female's life. Here, we tested whether oxidative stress is a consequence of reproduction in the brains of female Wistar rats. We evaluated the activities of glutathione peroxidase, glutathione S-transferase, and superoxide dismutase; H_2O_2 consumption; protein carbonylation; NO_2 & NO_3 levels; and total glutathione, as well as sex hormone levels in brain tissue of animals at 3, 6, 12, and 24 months of age. Animals were grouped according to reproductive experience: breeders or non-breeders. Most of the studied parameters showed a difference between non-breeders and breeders at 12 and 24 months. At 24 months of age, breeders showed higher superoxide dismutase activity, H_2O_2 consumption, glutathione peroxidase activity, and carbonyl levels than non-breeders. In 12-month-old non-breeders, we observed a higher level of H_2O_2 consumption and higher superoxide dismutase and glutathione peroxidase activities than breeders. By evaluating the correlation network, we found that there were a larger number of influential nodes and positive links in breeder animals than in non-breeders, indicating a greater number of redox changes in breeder animals. Here, we also demonstrated that the aging process caused higher oxidative damage and higher antioxidant defenses in the brains of breeder female rats at 24 months, suggesting that the reproduction process is costly, at least for the female brain. This study shows that there is a strong potential for a link between the cost of reproduction and oxidative stress.

1. Introduction

Reproduction is very costly to animals, and changes in cellular structure and function are likely to occur as a result (Pichaud et al., 2013). In mammals, females usually invest much more in parental care than males (Clutton-Brock, 1991), and lactation is the most energetically demanding period of a female's life. As a consequence, it has been predicted that oxidative stress could increase during this reproductive period (Speakman, 2008).

The most frequently suggested link connecting oxidative stress with reproduction involves the high levels of metabolism associated with reproductive investment (Garratt et al., 2013). The high metabolic effort associated with reproduction increases the production of damaging reactive oxygen and nitrogen species (ROS and RNS) that escape during metabolic processes (Bergeron et al., 2011). Although ROS and RNS play an important signaling role (Dickinson and Chang, 2011), they can also cause oxidative damage to biomolecules such as lipids, proteins, and DNA.

We currently know very little about how reproduction costs are actually incurred because the majority of studies have focused on the eventual outcomes rather than the proximate mechanisms (Costantini, 2014). It has been suggested that oxidative stress may be one key cellular mechanism underlying reproduction costs (Costantini, 2008; Metcalfe and Alonso-Alvarez, 2010; Monaghan et al., 2009). This hypothesis is being considered because a high investment in reproduction would result in faster somatic deterioration and reduced life expectancy because resources allocated for reproduction are no longer available for self-maintenance (Metcalfe and Monaghan, 2013). The few studies in which reproductive effort was manipulated, for example, found that enzymatic and non-enzymatic antioxidant defenses may be altered, possibly sacrificed in favor of investment in reproduction (Alonso-Alvarez et al., 2004; Losdat et al., 2011; Wiersma et al., 2004) or upregulated in response to an increase in free radical production (Garratt et al., 2013).

In animal societies, the competition for and acquisition of status may be very intense; hence, it may also carry significant fitness costs. In many cooperatively breeding mammals, a single female has a dominant status, and she prevents subordinate females from breeding (Solomon and French, 1997). Upon investigating oxidative stress in various tissues, the aforementioned studies also revealed that in some species,

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reproducing females are experiencing less oxidative damage than those that do not reproduce (Schmidt et al., 2014). Like social bees, ants, and termites, there is only one female in a Damaraland mole-rat colony that reproduces; all other female colony members are reproductively suppressed (Bennett et al., 2005; Remolina and Hughes, 2008) and the reproductive female lives longer than her non-reproductive counterparts (Schmidt et al., 2013). The reduction in oxidative damage in breeding females may be attributable to the unusual social structure of this species, as similar relationships have been observed between reproductive and non-reproductive eusocial insects (Aurori et al., 2014; Schrempf et al., 2011).

Aging can also be defined as a progressive functional decline, or a gradual deterioration of physiological function with age, including a decrease in fecundity (López-Otín et al., 2013). Many biological changes contribute to variability in brain aging, and the literature indicates an important impact of oxidative stress on brain deterioration among older individuals (Mariani et al., 2005; Muller et al., 2007). The brain is an aerobic organ that has one of the highest oxygen consumption rates on the basis of weight. Thus, the brain may be a tissue that is more susceptible to oxidative damage by free radicals (Haider et al., 2014).

Females live longer than males in many mammalian species, including humans (Borras et al., 2007; Vina et al., 2011), and show a lower incidence of several neurodegenerative diseases (Lee et al., 2008). A few studies on birds and mammals found that females tend to have lower levels of oxidative damage and higher levels of antioxidants (Casagrande et al., 2011; Vina et al., 2011). Female rats show more highly differentiated mitochondria, meaning greater mitochondrial machinery, and, as a result, the mitochondria of female rats show a higher capacity and efficiency for substrate oxidation than those of males in the liver (Valle et al., 2007), brown adipose tissue (Justo et al., 2005; Rodriguez-Cuenca et al., 2002), cardiac tissue (Colom et al., 2007), and brain (Guevara et al., 2009), which has been associated with greater mitochondrial functionality.

The trade-off between reproductive investment and lifespan is the single most important concept in life history theory. There is much evidence to support the existence of this trade-off, but the physiological costs of reproduction that underlie this relationship remain poorly understood. We have demonstrated previously that reproductive experiences alter the oxidative profile in the kidneys of female rats during aging (da Silva et al., 2013). The aim of this study is to examine how reproductive experiences alter the oxidative profile in the brains of female rats during aging.

2. Materials and methods

2.1. Animals

All animal studies followed the rules from the EU Directive for animal experiments 2010/63/EU and the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health (DHEW Publication No. (NIH) 85-23, revised in 1996, Office of Science and Health Reports, Division of Research Resources/NIH, Bethesda, MD, USA) and were approved by the Ethics Committee of the Universidade Federal do Rio Grande do Sul, Brazil. This study employed 80 Wistar female rats (*Rattus norvegicus*) aged three, six, twelve, and twenty-four months. At one month of age, rats were divided into two groups: with (breeders) or without (non-breeders) reproductive activity ($n = 10$ for each age and group). Breeders were maintained in a box with a single male of the same age (1 male and 1 female per box). Non-breeder rats were grouped with other female rats without any male contact (5 per box). Reproduction was considered to have occurred when the females gave birth to litters. Pups were separated from the couple at 21 days of age, i.e., before the initiation of the pubertal stage, which corresponds to an age of 30–70 days for males and 33–42 days for females (Krinke, 2000). Litter sizes ranged from 1 to 15 pups, and each couple had 5 to 15 litters, The females had litters until 12 months of age, and one had a litter at 16 months of age. The animal house was kept on a 12 h light/dark cycle at a temperature of 24 ± 1 °C, and animals were provided with standard lab chow and drinking water ad libitum. Vaginal smears were performed periodically to monitor the estrous cycle. Females were sacrificed at the proestrus stage up to 12 months of age, and the cycles of most females (95%) 24 months of age were stopped at the diestrus phase.

2.2. Brain dissection and processing

Animals were euthanized according to our experimental protocol when they reached three, six, twelve, or twenty-four months of age. All animals were anesthetized using a mixture of ketamine and xylazine (i.p.; 75 mg/kg and 10 mg/kg, respectively), and their body weight and length (without tail) were measured. After perfusion using a saline infusion, the brain was removed from the skull, weighed, and immediately frozen in liquid nitrogen for further analysis.

Organ processing was performed as described previously (Hackenhaar et al., 2009). Briefly, brains were processed with manual maceration. The samples were sonicated in 30 mmol/L phosphate buffer (120 mmol/L KCl, 100 μmol/L PMSF, phenylmethanesulfonylfluoride, pH 7.4) and centrifuged for 10 min at 3500*g*. The supernatant was transferred to a fresh tube, and a second centrifugation was performed for 10 min at 15,800*g*. The supernatant from the second centrifugation was used for all assays.

2.3. Sample collection

Before perfusion, blood was collected by puncturing the left ventricle of the heart. Fresh blood was centrifuged for 4 min at 3209*g*, and the serum was separated for subsequent immunoassays.

2.4. Estradiol level measurements

Levels of 17β-estradiol in the serum were measured by ELISA immunoassays using the Estradiol IBL International Tecan kit (Hamburg, Germany).

2.5. Assays for antioxidant molecules in brain tissue

Total GSH (tGSH) was determined by measuring the formation of p-nitrophenol from 5,5-dithiobis (2-nitrobenzoic acid) in the presence of glutathione reductase and NADPH (Kondo and Awada, 2000). Briefly, we used 20 μL of 0.1 mmol/L potassium phosphate to generate a calibration curve. For the assay, 12 μL of 5,5-dithiobis (2-nitrobenzoic acid) 0.1 mol/L plus glutathione reductase 6 U/mL were added to 20 μL of tissue extract; 60 μL of NADPH 4 mmol/L were added after 30 s. Color development was read at 412 nm, and the level is expressed as micromolar of glutathione per milligram of protein.

2.6. Assays for enzyme activities in brain tissue

To verify the enzymatic kinetic of GPx, one solution was prepared with buffer phosphate 100 mmol/L, pH 7.4, 1 mmol/L EDTA, 0.15 mmol/L NADPH, 1 mmol/L glutathione and 1 U glutathione reductase enzyme. The reaction was started with the addition of 10 μL of Tert-Butyl hydroperoxide (70%) and the GPx activity was measured by observation of the NADPH decrease with a spectrophotometer at 340 nm (Pinto and Bartley, 1969). The consumption of hydrogen peroxide (H_2O_2) as evaluated by measuring the rate of H_2O_2 consumption via absorbance at 240 nm (Aebi, 1984). The activity was expressed as units per milligram of protein; 1 U was defined as the capacity to consume 1 μmol of H_2O_2 per minute. We report consumption of H_2O_2 because there are multiple mechanisms of detoxification of H_2O_2 (mainly CAT and peroxiredoxins), and the test is not specific for any of them.