Experimental Gerontology 46 (2011) 773–780

Contents lists available at ScienceDirect

Experimental Gerontology

journal homepage: www.elsevier.com/locate/expgero

Oxidative stress in the kidney of reproductive male rats during aging

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ARTICLE INFO

Article history:
Received 3 December 2010
Received in revised form 6 April 2011
Accepted 12 May 2011
Available online 17 May 2011

Section Editor: Christiaan Leeuwenburgh

Keywords:
Reproduction
Antioxidant enzymes
Antioxidant compounds
Hormones
Oxidative damage

ABSTRACT

Reproduction alters the male physiology. We performed a comprehensive examination of oxidative stress in the kidneys of male rats with (experienced) or without (naïve) reproductive activity during aging. Oxidative stress was assessed by measuring the activity of catalase, glutathione peroxidase, glutathione S-transferase, and superoxide dismutase, and by measuring protein carbonylation, lipid peroxidation, nitrite and nitrate levels, vitamin C levels, and glutathione (total, reduced, and oxidized forms) levels, and metabolism was accessed by aconitase activity in kidney tissue, as well as testosterone and estradiol levels in serum. Reproductively active animals exhibited increased testosterone levels and altered metabolism. Aging affects tissues and organs and contributes to their functional decline. Elderly naïve rats showed high nitrite and nitrate levels. The experienced rats had less damage in elderly ages, probably because they had higher antioxidant amount and antioxidant enzyme activities at earlier ages, which would have avoided oxidative damage seen in naïve group, and because of the metabolism decline. Glutathione increase in naïve elder rats probably was induced for direct protection against oxidative damage and indirect protection by higher glutathione peroxidase and glutathione S-transferase activities. Linear regression shows that lipid peroxidation levels explained vitamin C levels (B standardized value of 0.42), indicating that vitamin C was properly produced or recruited into kidneys to combat lipid peroxidation. Catalase activity reflected the protein carbonylation and lipid peroxidation levels (B standardized values of 0.28 and 0.48). These results add comprehensive data regarding changes in oxidative stress during aging, and suggest an explanation for the costs of reproduction.

1. Introduction

Reproduction causes changes in male vertebrates, including morphological, behavioral, and physiological alterations (Miles et al., 2007). Reproductive success incurs some metabolic costs, and previous studies have suggested that reproduction causes oxidative stress (Alonso-Alvarez et al., 2004, 2007; Arenas-Rios et al., 2007; Harshman and Zera, 2007; Wang et al., 2001; Wiersma et al., 2004). Oxidative stress is related to aging (Harman, 1956) and is an important issue because aging is accompanied by an increased incidence of renal disease and associated morbidity, which pose a pressing problem in terms of public health and costs (Dowling and Simmons, 2009).

Most studies concerning reproductive activity and oxidative stress have been restricted to invertebrates such as *Drosophila melanogaster* (Harshman and Zera, 2007; Wang et al., 2001) or *Caenorhabditis elegans* (Harshman and Zera, 2007), and few studies have addressed vertebrates (Alonso-Alvarez et al., 2004; Arenas-Rios et al., 2007; Costantini, 2008; Wiersma et al., 2004; Dowling and Simmons, 2009). Studies on vertebrates have typically been limited either to females (lactation, number of litters, size of litter) (Speakman, 2008), or have related to male behavior (Hull and Dominguez, 2007). We recently reported an analysis of brains of reproductive male rats during aging, which is, to our knowledge, the only study focusing on aging and oxidative stress in breeding male mammals (Alabarse et al., 2011). In this study, using controlled conditions for temperature, food access, and light/dark cycle, we identified changes in rat brain associated with oxidative stress accompanying reproductive experience.

Reactive oxygen species (ROS), which can be produced by mitochondria and peroxisomes, can cause oxidative damage including protein modification and lipid peroxidation (Abegg et al., 2010; Gobe and Crane, 2010; Gredilla and Barja, 2005; Guevara et al., 2009; Herlein et al., 2009; Razmara et al., 2007). Inhibition of ROS production may protect cells from dysfunction and may prevent cell death. This has been important in kidney research where studies on antioxidants are focused on developing therapeutic strategies to prevent renal cell oxidative damage (Gobe and Crane, 2010; Jankauskas et al., 2010). Among these are studies of metal-induced renal alterations related to oxidative stress produced by exposure to cadmium, lead, selenium, iron, and zinc (Gobe and Crane, 2010;

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doi:10.1016/j.exger.2011.05.006

Abdallah et al., 2010; Matsumoto et al., 2009). These metals preferentially accumulate in kidney, which is the primary site of toxicity (Gobe and Crane, 2010; Matsumoto et al., 2009). Metals may alter enzyme activities, lower glutathione and other antioxidant reserves in the body, and disrupt calcium homeostasis (Gobe and Crane, 2010; Abdallah et al., 2010).

In males, steroid hormone concentrations change during aging and as a consequence of reproductive behavior. Male behavioral stress during reproductive activity can be caused by many factors such as (i) social stress related to contact with female and pups, (ii) physical stress during sexual acts and resulting from female aggressiveness, and (iii) physiological stress associated with hormonal and metabolic changes that reproduction causes. All of these factors can result in oxidative stress.

Changes in sex steroid hormones in a breeding male may alter the biochemical balance of the kidney. Testosterone is known to have multiple activities; it is considered a pro-oxidant and a promoter of renal crystal deposition and urolithiasis; it increases 8-hydroxydeoxyguanosine (DNA damage marker) urinary levels; it can act indirectly to stimulate sodium reabsorption, which increases systolic blood pressure, thus contributing to hypertension and renal failure. Estradiol, on the other hand, is frequently addressed as an antioxidant, an inhibitor of renal crystal deposition and urolithiasis, and decreases 8-hydroxydeoxyguanosine urinary levels (Halliwell and Gutteridge, 2007; Perez-Torres et al., 2010; Yi et al., 2009; Yoshioka et al., 2010; Reckelhoff et al., 2005). Both of these hormones are potential pharmacological and therapeutic targets for treatment of renal disorders (Irnaten et al., 2009).

Aconitase (ACO) activity has a role in controlling oxidative processes in the cell including the functioning of the tricarboxylic acid cycle, resulting in induction of oxidation of the electron transport chain components – O_2 to O_2– reducers, and its activity represents the mitochondrial metabolic status (Medvedeva et al., 2002). Mitochondrial ROS production is also associated with aging through mechanisms related to oxidative damage to biomolecules which may disrupt signal transduction pathways (Sastre et al., 2003; Vina et al., 2009). Antioxidant defenses can retard free radical associated aging (Harman, 1956; Hackenhaar et al., 2009). Antioxidant enzymes act on reactive species, as can be listed: catalase (CAT), glutathione peroxidase (GPx), glutathione S-transferase (GST), and superoxide dismutase (SOD). Among non-enzymatic antioxidant defenses available to cells, reduced glutathione is primary. Vitamins, *e.g.*, vitamin C (Vit C), can also protect against oxidative stress (Adibhatla and Hatcher, 2010).

The knowledge upon oxidative stress and reproductive activity, the kidney age-related functional decline, and the researches weakness on aging treatments suggests the need of one deep study in the basis of kidney oxidative status that could clarify ways to protect it against oxidative stress and lead it to better functions during aging.

In view of the fact that oxidative stress occurs during reproduction and during aging, the aim of this study was to evaluate the levels of hormones that alter metabolism during reproduction and aging, and examine oxidative stress parameters, such as antioxidant enzymes and compounds, and oxidative damage markers in kidneys of male rats that were reproductively active (experienced) or reproductively inactive (naïve) during aging. This analysis represents a logical extension of our previous work (Alabarse et al., 2011), which focused on alterations in brains of breeding males during aging.

2. Material and methods

2.1. Chemicals

Ketamine, xylazine, NaCl, KCl, Na_2HPO_4, NaH_2PO_4, PMSF, KH_2PO_4, $MgCl_2$, hexaamminecobalt (III) chloride, tris base, sodium citrate, ascorbic acid, $NaNO_2$, sulfanilamide, KOH, HCl, perchloric acid, guanidine hydrochloride, 1-chloro-2,4,-dinitrobenzene, epinephrine, glycine, NADPH, $NADP^+$, reduced glutathione, BSA, isocitrate dehydrogenase from *Bacillus subtilis*, glutathione reductase from *Saccharomyces cerevisiae*, tert-butyl hydroperoxide, hydrogen peroxide, EDTA, Griess reagent, methanol, ethanol, ethyl acetate, 5,5-dithiobis (2-nitrobenzoic acid), *N*-ethylmaleimide, 2,4-dinitrophenylhydrazine, trichloroacetic acid, 1,1,3,3-tetraethoxypropane, EDTA, and Bradford reagent, were obtained from Sigma-Aldrich.

2.2. Equipments

Radioimmunoassays were performed with a Gamma Counter C12 (DPC Medlab, CA, USA), enzyme kinetic assays were performed with a Femto Spectrophotometer 800XI (Femto Indústria e Comércio de Instrumentos, SP, Brazil), and nonkinetic assays were performed with a Softmax Tunable Microplate Reader (Molecular Device Corporation, CA, USA), liquid chromatography assays were performed with a Shimadzu HPLC equipment with SPD-20A UV–visible detector and LC-20AT (Shimadzu Corporation, Kyoto, Japan).

2.3. Animals

All animals and all animal care and grouping were the same as previously published (Alabarse et al., 2011). These studies were approved by the Animal Ethics Committee of the Universidade Federal do Rio Grande do Sul, Brazil. This study employed 80 Wistar male rats (*Rattus norvegicus*) aged three, six, twelve, and 24 months. At 1 month of age rats were divided into two groups: experienced and naïve ($n = 10$ for each age and group). Experienced rats were maintained in a box with a single female of the same age (1 male and 1 female per box). Naïve rats were grouped with other male rats without any contact with females (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.*, prior to onset of puberty, which corresponds to an age of 30–70 days for males and 33–42 days for females (Krinke, 2000). Litter size ranged from 3 to 11 pups, and each couple had 8 to 12 litters.

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*.

2.4. Obtaining kidneys and processing

Animals were sacrificed according to the experimental protocol when they reached three, six, twelve, or 24 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 body weight and length (without tail) were measured. After saline perfusion, the kidneys were removed as previously described (Hackenhaar et al., 2009), weighed, and immediately frozen in liquid nitrogen for further analysis. Briefly, both kidneys of each animal were processed together with manual maceration. The samples were sonicated in 30 mM phosphate buffer (120 mM KCl, 100 mM PMSF, 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.

2.5. Obtaining blood

Before perfusion, blood was quickly collected by puncturing the left ventricle of the heart. Fresh blood was centrifuged for 4 min at 320 *g*, and the serum was separated for subsequent radioimmunoassay.