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Original Research Article

Effect of neonatal or adult heat acclimation on testicular and epididymal morphometry and sperm production in rats

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ARTICLE INFO

Article history:
Received 3 November 2014
Received in revised form
3 January 2015
Accepted 9 January 2015
Available online 21 January 2015

Keywords:
Heat acclimation
Testis
Epididymis
Sperm motility
Sperm concentration

ABSTRACT

The accessory gland weight, testicular and epididymal morphometry and sperm production were analyzed in four groups of rats housed at 20 or 34 °C: (1) control rats (CR) kept at 20 °C from birth to day 90; (2) adult heat-acclimated rats (AHA) kept at 20 °C from birth to day 45 followed by 34 °C to day 90; (3) neonatal heat-acclimated rats (NHA) kept at 34 °C from birth to day 90 and (4) de-acclimated rats (DA) kept at 34 °C from birth to day 45 followed by 20 °C to day 90. In NHA and DA rats, accessory gland weight was higher than in controls. Despite the lack of differences in testicular and epididymal morphometry, curvilinear velocity of spermatozoa was lower in the NHA group compared to controls. Areas of seminiferous tubules were lower in the DA than in CR and NHA groups, however, sperm concentration and motility were not affected by the treatment in this group. In AHA rats, epithelium of approximately 20% of seminiferous tubules was degenerated and Sertoli cell number was lower in the remaining tubules. In contrast to sperm motility, epididymal duct area, area of the duct occupied by spermatozoa and cauda epididymis sperm concentration were lower in AHA rats than in the other groups. In conclusion, neonatal heat acclimation did not affect the testicular morphometry and epididymal sperm concentration, suggesting adjustment to high ambient temperature. On the contrary, adult heat acclimation of rats affected the examined parameters, leading to decreased sperm concentration.

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http://dx.doi.org/10.1016/j.repbio.2015.01.001

1. Introduction

Seminiferous tubule abnormal morphology, lower spermatozoa concentration and motility as well as lower sperm fertilizing ability are frequently observed in males exposed to high-temperature [1–3]. Most studies concentrate on the effect of acute heat shock applied directly to the testes [1–3]. Studies on the long-term effect of increased body temperature on sperm production in mammals are rare [4], but they showed that long-term heat acclimation is less deleterious for testicular function then acute heat shock. Moreover, it was demonstrated that non-reproductive parameters (i.e., physiological and transcriptomic) differ between short- and long-term heat acclimated rats [5,6]. Short-term heat treatment induces activation of stress response genes; such activation, however, is not observed after long-term heat acclimation [6]. It seems that both the short- and long-term study models should be employed by researchers to fully respond to human as well as farm and wild animal reproductive problems [3,7]. Since reproductive processes undergo dynamic changes during postnatal life of mammals, the starting time-point of the heat exposure determines the organism response to the trial [8]. In contrast to other systems that are not fully differentiated at birth [9], the developmental differences in the adaptive ability of testicular function to high temperature were not examined.

We have previously shown that high ambient temperature applied to newborn male rats distinctly affected the functioning of the pituitary–testicular axis compared to adult males exposed to the same thermal conditions [10]. We found better functional adaptation of this axis to high ambient temperature in neonatal than in adult heat-acclimated rats. Thus, we also expected differences between neonatal and adult heat-acclimated rats in morphometry of the seminiferous tubules and the epididymal duct as well as in the production and maturation of spermatozoa. Therefore, we examined the effects of high housing temperature on: (1) reproductive organ weight, (2) morphometry of seminiferous tubules in temperature dependent (II–V, XI–XIV) and androgen dependent (VIII–IX) stages of the seminiferous epithelium cycle, (3) morphometry of epididymis, and (4) epididymal spermatozoa content and motility in neonatal and adult heat-acclimated rats.

2. Materials and methods

2.1. Experimental animals

Twenty two male Wistar rats were divided into four groups: (1) control males (CR, $n = 6$), born and housed until 90 day of life at 20 ± 1 °C; (2) adult heat-acclimated males (AHA, $n = 6$), born at 20 ± 1 °C and at 45 day of the postnatal life subjected to 34 ± 1 °C for the next 45 days; (3) neonatal heat-acclimated males (NHA, $n = 5$), born and housed at 34 ± 1 °C for 90 days, and (4) deacclimated males (DA, $n = 5$), housed at 34 ± 1 °C from birth to 45 day, and then at 20 ± 1 °C for another 45 days. Both, the humidity and light:dark cycle (12:12 h) were controlled in the experiment. The rats had free access to tap water and chow pellets (Labofeed H; Feeds and Concentrates Production Plant, Kcynia, Poland). At 90 day of the postnatal life, all rats were sacrificed by cervical dislocation, and whole testes, epididymides, seminal vesicles and ventral prostates were collected. Local Ethic Committee at the University of Warmia and Mazury in Olsztyn approved the experiments (NO 46/2008/N).

2.2. Body and relative weight of reproductive organs

Because body weight was lower in all heat-acclimated groups (AHA, NHA and DA) than in CR group, the testes weight was determined as gonado-somatic index (GSI) [10] and the weight of other reproductive organs as relative to the body weight. The weight of the testes and sex accessory glands (epididymis, seminal vesicles) is presented as a paired organ weight.

2.3. Morphometry of the testes and epididymides

For morphometrical analysis, testicular *tunica albuginea* was incised and the right testicles were placed in 10% buffered formaldehyde. The right epididymis was cleared from adjacent fat pad, followed by caput, corpus and cauda separation and formaldehyde fixation. Fixed testes and each part of epididymides were processed to obtain paraffin blocs and cut to 7 μm serial sections. Every tenth testicular section was stained with hematoxylin and eosin (POCH, Gliwice, Poland). Epididymal sections were stained by periodic acid-Schiff reaction [11].

Sections of reproductive organs were then subjected to morphometrical analysis, using calibrated photomicrographs (Olympus, Tokyo, Japan) of testicular seminiferous tubules and seven parts of epididymal duct: three segments of caput, one segment of corpus and three segments of cauda epididymis. Image analysis software (AnalySis, Olympus) was used to obtain morphometric data. The stages II–V, VIII–IX and XI–XIV of the seminiferous epithelium cycle were recognized according to Hess [12]. The border of the tubule and tubular lumen in testicular as well as epididymal cross-sections were marked to measure the tubular or lumen area and radius. The height of epithelium was then calculated as tubular radius minus lumen radius. Thirty measurements (three different cross-section, $n = 10$/cross-section) were taken from each epididymal part, and similar procedure was applied to the testis at every developmental stage.

Sertoli cell counts were done by Sertoli cell nuclei point counting in tubular cross-section from the VIII stage of the seminiferous epithelium cycle and were repeated to obtain 30 counts from three different cross-sections per testis [13].

2.4. Cauda epididymis spermatozoa number and motility

Left cauda epididymis from each rat was cut into pieces, placed in 3 mL M199 medium supplemented with 1% BSA and incubated in water bath at 34 °C for 5 min [14]. Fifty μL of spermatozoa suspension was transferred into new tube and diluted with the fresh medium for sperm motility determination. Triton X100 (0.05%) was added to the rest of suspension and homogenized for spermatozoa count, based on homogenization-resistant heads of mature spermatozoa [15]. The homogenate was 20 times diluted with phosphate buffer (pH 7.4) and spermatozoa were counted in hemocytometer in duplicate.