FOLIA HISTOCHEMICA ET CYTOBIOLOGICA
Vol. 47, No. 2, 2009
pp. 215-219

Changes in ambient temperature and oxygenation during the proestrus do not affect duration, regularity and repeatability of the estrus cycle in female rats

Arkadiusz Baran[1], Grzegorz Jakiel[2], Grażyna Wójcik[1]

[1]Department of Human Physiology, Medical University of Lublin,
[2]Department of Reproduction and Andrology, Medical University of Lublin,

Abstract: There are a lot of factors affecting the release of hormones from the anterior part of pituitary gland and their interactions with other parts of the endocrine, nervous and immune systems. The special significance of the proestrus phase of the estrous cycle of the rat, during which LH and FSH levels increase, followed by ovulation is known. The short length of the estrous cycle and the well recognized sequence of vaginal lavage cytology make it useful for investigating the influence of a stressful environment on the reproductive function. Short duration and mild changes in environmental conditions is considered as a factor analogous to psychological stress. The study was undertaken to determine the effects of a short duration change in the ambient temperature and oxygenation (30 minutes) on the proestrus phase of reproductive cycle and on the repeatability and regularity of phases of the reproductive cycle of Wistar strain rats. The animals were kept under standard conditions and had food and water available ad libitum. The climatic chamber with automatically adjustable and monitored internal parameters (temperature, oxygenation, humidity) was used to develop stress conditions. An estimation of the vaginal lavage using the microscope was done to determine the estrous cycle. The animals were divided into 6 groups. On the day of experiment: the control group (CG) stayed in the climatic chamber for 30 minutes (ambient temperature 21°C, normoxia – 21% O_2), the five test groups (TG – I – V) remained in the climatic chamber for 30 minutes, in the established environmental conditions (I – 21°C, 10% O_2; II – 10°C, 21% O_2; III – 10°C, 10% O_2; IV – 35°C, 21% O_2; V – 35°C, 10% O_2). During the following days after the experiment, a microscopic estimation of vaginal lavage was collected over again. There were no changes of duration and sequence of the present estrous cycle and repeatability of the next cycles. Our results indicate that short duration change in the ambient conditions do not cause a disturbance in the hypothalamo – pituitary – gonadal axis, although it can activate adaptation mechanisms of the organism.

Key words: temperature, oxygenation, reproductive cycle, climatic chamber, rat

Introduction

There are a lot of factors affecting the release of hormones from the anterior part of pituitary gland and their interactions with other parts of the endocrine, nervous and immune systems. The special significance of the proestrus phase of the estrous cycle of the rat, during which LH and FSH levels increase and then are followed by ovulation is known. Short duration and mild changes in environmental conditions is considered as a factor analogous to psychological stress. Reproductive activity is one of the main functions that becomes altered and is inactivated during the adaptive response to stress [1]. Chronic exposure to stressors increases the hypothalamic – pituitary – adrenal (HPA) axis activity and concomitantly reduces hypothalamic – pituitary – gonadal (HPG) axis activity. This antagonistic relationship between both of these axes has been proposed to underlie the inhibition of reproductive function due to stress [2]. The short length of the estrous cycle of rats makes them ideal for investigating changes occurring during the reproductive cycle. The estrous cycle lasts some days and is composed of: proestrus, estrus, metestrus and diestrus phases, which may be determined according to the cell types observed in a vaginal smear. A proestrus smear consists of a predominance of nucleated epithelial cells; an estrous smear primarily consists of anucleated cornified cells; a metestrous smear consists of the same proportion among

Correspondence: A. Baran, Dept. of Human Physiology, Medical University of Lublin, Radziwiłłowska 11, 20-080 Lublin, Poland; tel./fax: (+4881) 5288405, e-mail: arkadiusz-baran@go2.pl

Folia Histochem Cytobiol. 2009:47(2): 215 (215-219)
doi: 10.2478/v10042-009-0017-z

Table 1. The duration (means ± SD), regularity and/or repeatability of the estrous cycles in rats, before, during (except repeatability) and after the acclimation in the climatic chamber. Regularity means that the sequence of the estrous cycle is following: proestrus, estrus, metestrus and diestrus. Repeatability means that the next cycle has the same sequence of phases. Statistical comparison of the data was done by ANOVA. There were no significant differences ($p>0.05$).

Before the acclimation in the climatic chamber					
	number of observed cycles	mean duration of estrous cycle (days)	± SD	regularity of the estrous cycles	repeatability of the following cycles
control					
CG – 21°C, 21% O_2	17	4.06	0.24	yes	yes
test groups					
TG - I – 21°C, 10% O_2	17	4.06	0.24	yes	yes
TG - II – 10°C, 21% O_2	17	4.06	0.24	yes	yes
TG - III – 10°C, 10% O_2	17	4.06	0.24	yes	yes
TG - IV – 35°C, 21% O_2	17	4.06	0.24	yes	yes
TG - V – 35°C, 10% O_2	17	4.06	0.24	yes	yes
The estrous cycle with acclimation in the climatic chamber					
	number of observed cycles	mean duration of estrous cycle (days)	± SD	regularity of the present estrous cycle	
control					
CG – 21°C, 21% O_2	1	4	0	yes	
test groups					
TG - I – 21°C, 10% O_2	1	4	0	yes	
TG - II – 10°C, 21% O_2	1	4	0	yes	
TG - III – 10°C, 10% O_2	1	4	0	yes	
TG - IV – 35°C, 21% O_2	1	4	0	yes	
TG - V – 35°C, 10% O_2	1	4	0	yes	
After the acclimation in the climatic chamber					
	number of observed cycles	mean duration of estrous cycle (days)	± SD	regularity of the estrous cycles	repeatability of the following cycles
control					
CG – 21°C, 21% O_2	2	4	0	yes	yes
test groups					
TG - I – 21°C, 10% O_2	2	4	0	yes	yes
TG - II – 10°C, 21% O_2	2	4	0	yes	yes
TG - III – 10°C, 10% O_2	2	4	0	yes	yes
TG - IV – 35°C, 21% O_2	2	4	0	yes	yes
TG - V – 35°C, 10% O_2	2	4	0	yes	yes

leukocytes, cornified, and nucleated epithelial cells, and a diestrous smear primarily consists of a predominance of leukocytes [3]. Ovulation occurs from the beginning of proestrus to the end of estrus phase [4,5]. From the onset of sexual maturity up to the age of 12 months, the mean cycle length in the female rat is 4 days [6-8]. During the estrous cycle, prolactin, LH and FSH remain low and increase in the afternoon of the proestrus phase. Estradiol levels begin to increase during metestrus, reaching peak levels during proestrus and returning to baseline levels during estrus. Progesterone secretion also increases during the metestrus and diestrus phases followed by a decline. The progesterone value rises to reach its sec-

doi: 10.2478/v10042-009-0017-z

ond peak towards the end of proestrus [7,9]. Vaginal secretions from female rats could be collected every morning and unstained native material could be observed using a microscope without the aid of a condenser lens. The collection of vaginal lavage from the animals, the observation of the material in the microscope and the determination of the estrous cycle phase of the female rats are all quick procedures. In studies about the reproductive system as well as studies about the influence of the estrous cycle on non-reproductive functions [8,10-12], vaginal smear cytology is used for the determination of the estrous cycle phases. Studies in rats, mice and humans have shown that acute exposure (from seconds to a few hours) to stressors such as immobilization, electric foot shock, cold, exercise, food restriction or to situations that cause anxiety is characterized by an increase in corticotrophin releasing hormone (CRH), adrenocorticotropin (ACTH), beta-endorfins, and corticosterone in rodents and cortisol in humans [13]. Conversely, acute stress by immobilization decreases serum levels of luteinizing hormone (LH) and testosterone [14], or do not modify their levels [15]. In intact rats, acute exposure to stress from noise or water immersion, immobilization, cold, heat, light or surgery stimulates the HPG axis [16,17], increasing LH and follicle stimulating hormone (FSH), prolactin and testosterone levels in the plasma of stressed males. On the other hand, chronic stress by immobilization, intermittent electric foot shocks, prolonged exercise, constant illumination, forced swimming in cold water, noise, surgery, crowding and social stress in rats [2], causes a decrease in hypothalamic CRH content, an increase in plasma levels of ACTH and glucocorticoids, as well as a general inhibitory effect on HPG axis, through decreasing LH and testosterone [13].

Mammals display an ability to adjust their physiological mechanisms to overcome shifts in their environment. This process, defined as acclimation, usually involves alterations in the capacity and responsiveness of pre-existing features that are beneficial to the organism in the new environment. Thus, during heat or cold acclimation both central and peripheral modifications enhance the ability of thermoregulatory effectors to cope with thermal stress [18]. The heat acclimation process involves two distinct phases: the first, short – term acclimation is an early, transient phase, characterized by an augmented autonomic signal output ratio to compensate readily for cellular perturbations. The second is a sustained stable phase called long-term heat acclimation, during which a new homeostasis is achieved [19,20]. Hypoxia is a condition of decreased oxygen (O_2) levels that elicits homeostatic responses aimed at counteracting the negative effects of O_2 depletion [21]. Hypoxia induces the expression of several genes, such as erythropoietin [22], vascular endothelial growth factor [23], and other agents that favor the adaptation of the organism to the decreased availability of O_2. Hypoxia is an essential developmental and physiological stimulus that plays a key role in the pathophysiology of heart disease, cancer, neuron death, cerebrovascular disease and chronic lung disease, which are the most common cause of mortality in Western nations [21,23]. These findings prompted our interest in the acclimatization processes and possibly influence of these factors on reproductive function of the female rat.

Materials and methods

Animals. Female Wistar rats (*Rattus norvergicus*), three months old, weighing 200 to 300 g were used. The animals were housed in standard cages, six per cage, in a controlled temperature room (21°C ± 1°C), with a 12 h light: 12 h dark cycle, lights on at 6:00 a.m. Standard laboratory chow and tap water were available *ad libitum*.

Breeding conditions. On the day of the experiment (proestrous phase of the cycle) animals were divided into 6 groups and acclimated in the established conditions. Control group – CG (n = 6) (21°C, 21 % O_2); test group TG – I (n=6) (21°C, 10 % O_2); test group TG – II (n=6) (10°C, 21 % O_2); test group TG – III (n=6) (10°C, 10 % O_2); test group TG – IV (n=6) (35°C, 21 % O_2); test group TG – V (n=6) (35°C, 10 % O_2). The animals remained in the climatic chamber for 30 minutes. The animals were tested once at a time in the climatic chamber [24,25]. The climatic chamber (Multiserv model KBI – 100, Lublin, Poland) is a box with plexiglas walls of dimensions 50 × 40 × 40 cm. Internal environmental parameters (oxygenation, temperature, humidity) are automatically adjustable and monitored. The acclimation in the climatic chamber started at 12 a.m. After the experiment animals were returned to their cages. On the next day after the experiment the estimation of the vaginal lavage using a microscope was done to determine the estrous cycle once again. The estimation of estrous cycle lasts for the next two consecutive cycles.

Estrus cycle assessment. During three months, every morning between 8:00 and 9:00 a.m. each animal cage was carried to the experimental room. The estimation of the vaginal lavage using a microscope was done to determine the estrous cycle. Unstained material was observed under a light microscope, without the use of the condenser lens, with 10 and ×40 objective lenses. Three types of cells could be recognized: round and nucleated ones are epithelial cells; irregular ones without nucleus are the cornified cells; and the little round ones are the leukocytes. The proportion among them was used for the determination of the estrous cycle phases [3,8].

Ethical issues. The experimental protocol was approved by the Ethical Committee on Human and Animal Experimentation of The Medical University of Lublin.

Statistical analysis. Data obtained in this study are expressed as mean values and standard deviation (±SD). Measurements were tested with a non-parametric one-way analysis of variance. The level of significance was set at 0.05.

Results

The mean duration of the estrous cycle before the acclimation in the climatic chamber was 4.06 days for female rats in all tested groups and did not differ from

Folia Histochem Cytobiol. 2009:47(2): 217 (215-219)
doi: 10.2478/v10042-009-0017-z

the mean duration of the estrous cycle of the control group (p>0.05) (Table 1). There were no significant changes in duration and sequence of the estrous cycle and repeatability of the next cycles after the acclimation of the animal in the climatic chamber (p>0.05). The mean duration of the estrous cycle after the experiment was 4 days.

Discussion

Despite the number of reports of stressors influencing the secretion of gonadotropins and gonadal steroids in rats, only a few of them have expressed a correlation between an increase in adrenal corticosteroids and the suppression of testosterone and reproductive function [2]. The results of some studies [13], show that reproductive function in rats is modified differentially, depending on the characteristics of each stressor and on the duration of exposure (acute or chronic). It has been proposed that the suppression of reproduction during stress could be caused by the effect of several hormones secreted during stress, such as CRH, ACTH, beta-endorfin, and glucocorticoids on HPG axis function [2]. Although the mechanisms for these effects on reproductive function are not fully elucidated, possible sites of acton include: a centrally mediated inhibition of gonadotropin – releasing hormone (GnRH) release by CRH, opioids and glucocorticoids, a glucocorticoid – mediated decrease in pituitary responsiveness to GnRH, resulting in decreased LH secretion; direct gonadal effects of glucocorticoids with subsequent alterations in sex steroid output; glucocorticoid – induced sex steroid target tissue resistance to gonadal sex steroids [26].

The neuro – immune – endocrine interface is mediated by cytokines as well. They act as auto/paracrine or endocrine factors regulating pituitary development, cell proliferation, hormone secretion and feedback control of the HPA axis. Soluble products that appear to transmit information from the immune compartments to the central nervous system (CNS) act as immunotransmitters and function in immunomodulatory neuroendocrine circuits. There is now cumulating evidence that there are important interactions between the immune and neuroendocrine systems, which may explain, in part some of the effects on adrenal, reproductive, growth and thyroid functions which occur in the pathophysiology of acute and chronic disease.

Our results confirmed that the mean duration of the estrous cycle of the rat is 4 days. The short period of climatic stress do not influence, the estrous cycle of female rats. There were no changes in duration and sequence of the next cycles after the acclimation of the animal in the climatic chamber. The mean duration of the estrous cycle after the experiment was 4 days. There were no irregular cycles, characterized by staying in the same phase during the 4-5 days did not occur. Cycles, in which an alternation among the phases didn't follow the sequence proestrus, estrus, metestrus and diestrus also did not occur. The results suggest that short period climatic stress does not interfere with reproductive functions of female rat if they occur during the proestrus phase of the estrous cycle. If any experiment is performed one or more days before the proestrous phase in a regular cycling rat, it can alter the estrous cycle and one can no longer guarantee that the next proestrus will occur on the expected day and the researcher can lose the animal [27]. If it is done in the late morning of the proestrous phase after the pool of releasable GnRH has been synthesized and before the time of triggering the gonadotropin surges, which occurs around 12 a.m. it will not change the sequence and repeatability of the estrous cycle [28].

The process of acclimation also induces non-thermoregulatory benefits. Among these, cross-tolerance against perturbations in oxygen supply/demand balance, including insults such as ischemia or hypoxia, have been thoroughly studied in rat's heart and brain. The "switched on" state of intracellular pathways, caused by acclimation, allows their rapid activation when subjected to the multi-modal stress [29]. It is noteworthy that heat acclimation induces cross-tolerance against several other environmental stressors, all demanding similar protective pathways. The process of acclimation involves changes in all levels of body organization including reprogramming of gene expression. Our results indicate that short duration change of the ambient conditions does not cause the disturbance of hypothalamo – pituitary – gonadal axis, although can activate adaptation mechanisms of the organism.

Acknowledgements: This study was supported by grant from Medical University of Lublin (PW 224/06).

References

[1] Johnson E. Mechanisms of stress: a dynamic overview of hormonal and behavioral homeostasis. *Neurosci Biobehav Rev*. 1992;16:115-130.

[2] Retana-Marquez S, Bonilla-Jaime H, Vazquez-Palacios G, Martinez-Garcia R, Velazquez-Moctezuma J. Changes in masculine sexual baehavior, corticosterone and testosterone in response to acute and chronic stress in male rats. *Horm Behav*. 2003;44:327-337.

[3] Mandl A.M. The phases of the oestrous cycle in the adult white rat. *J Exp Biol*. 1951;28:584-588.

[4] McClintock JA, Schwartz NB. Changes in pituitary and plasma follicle stimulating hormone concentrations during the rat estrous cycle. *Endocrinology*. 1968;83:433-441.

[5] Schwartz NB, Bartosik D. Changes in pituitary LH content during the rat estrous cycle. *Endocrinology*. 1962;71:756-762.

[6] Freeman ME, Reichert LE, Jr., Neill JD. Regulation of the proestrus surge of prolactin secretion by gonadotropin and

doi: 10.2478/v10042-009-0017-z

estrogens in the rat. *Endocrinology*. 1972;90:232-238.
[7] Spornitz UM, Dravid A, Winkelmann A. Scanning electron microscopic demonstration of surface and subsurface enzymes in rat endometrium during the estrous cycle. *Scanning*. 1998;20:224-234.
[8] Marcondes FK, Miguel KJ, Melo LL, Spadari-Bratfisch RC. Estrous cycle influences the response of female rats in the elevated plus-maze test. *Physiol Behav*. 2001;74:435-440.
[9] Smith MS, Freeman ME, Neill JD. The control of progesterone secretion during the estrous cycle and early pseudopregnancy in the rat: prolactin, gonadotropin and steroid levels associated with rescue of the corpus luteum of pseudopregnancy. *Endocrinology*. 1975;96:219-226.
[10] Moura MJ, Marcondes FK. Influence of estradiol and progesterone on the sensitivity of rat thoracic aorta to noradrenaline. *Life Sci*. 2001;68:881-888.
[11] Marcondes FK. Stress-induced subsensitivity to catecholamines depends on the estrous cycle. *Can J Physiol Pharmacol*. 1996;74:663-669.
[12] Rodrigues ML, Marcondes FK, Spadari-Bratfisch RC. Relationship among sensitivity to adrenaline, plasma corticosterone level and estrous cycle in rats. *Can J Physiol Pharmacol*. 1995;73:602-607.
[13] Retana-Marquez S, Salazar ED, Velazquez-Moctezuma J. Effect of acute and chronic stress on masculine sexual behavior in the rat. *Psychoneuroendocrinology*. 1996;21:39-50.
[14] Walker CD, Sapolsky RM, Meaney MJ, Vale WW, Rivier CL. Increased pituitary sensitivity to glucocorticoid feedback during the stress nonresponsive period in the neonatal rat. *Endocrinology*. 1986;119:1816-1821.
[15] Tsopanakis C, Tesserommatis C. Cold swimming stress: effects on serum lipids, lipoproteins and LCAT activity in male and female rats. *Pharmacol Biochem Behav*. 1991;38:813-816.
[16] Marti O, Gavalda A, Jolin T, Armario A. Acute stress attenuates but does not abolish circadian rhythmicity of serum thyrotrophin and growth hormone in the rat. *Eur J Endocrinol*. 1996;135:703-708.
[17] Siegel RA, Weidenfeld J, Feldman S, Conforti N, Chowers I. Neural pathways mediating basal and stress-induced secretion of luteinizing hormone, follicle-stimulating hormone, and testosterone in the rat. *Endocrinology*. 1981;108:2302-2307.
[18] Schwimmer H, Gerstberger R, Horowitz M. Heat acclimation affects the neuromodulatory role of AngII and nitric oxide during combined heat and hypohydration stress. *Brain Res Mol Brain Res*. 2004;130:95-108.
[19] Horowitz M. Do Cellular Heat Acclimation Responses Modulate Central Thermoregulatory Activity? *News Physiol Sci*. 1998;13:218-225.
[20] Horowitz M, Kaspler P, Simon E, Gerstberger R. Heat acclimation and hypohydration: involvement of central angiotensin II receptors in thermoregulation. *Am J Physiol*. 1999;277:R47-R55.
[21] Jeong HJ, Hong SH, Park RK, Shin T, An NH, Kim HM. Hypoxia-induced IL-6 production is associated with activation of MAP kinase, HIF-1, and NF-kappaB on HEI-OC1 cells. *Hear Res*. 2005;207:59-67.
[22] Jelkmann W, Hellwig-Burgel T. Biology of erythropoietin. *Adv Exp Med Biol*. 2001;502:169-187.
[23] Goldberg MA, Schneider TJ. Similarities between the oxygen-sensing mechanisms regulating the expression of vascular endothelial growth factor and erythropoietin. *J Biol Chem*. 1994;269:4355-4359.
[24] Baran A, Jankiewicz M, Wójcik G, Borowicz B, Jakiel G. Short stay in ambient hypoxia increases plasma interleukin-6 level in mice. *Pol J Environ Stud*. 2006;15-3A:55-58.
[25] Baran A, Jankiewicz M, Wójcik G, Borowicz B, Jakiel G. Temperature-induced increase of plasma interleukin 6 in mice during short period of adaptation to novel climatic conditions. *Pol J Environ Stud*. 2006;15-3A:148-150.
[26] Belda X, Marquez C, Armario A. Long-term effects of a single exposure to stress in adult rats on behavior and hypothalamic-pituitary-adrenal responsiveness: comparison of two outbred rat strains. *Behav Brain Res*. 2004;154:399-408.
[27] Poletini MO, Szawka RE, Freitas Marcon RM, Veiga MD, Franci CR, Anselmo-Franci JA. A method to study preovulatory surges of gonadotropins. *Brain Res Brain Res Protoc*. 2003;12:41-48.
[28] Levine JE. New concepts of the neuroendocrine regulation of gonadotropin surges in rats. *Biol Reprod*. 1997;56:293-302.
[29] Horowitz M, Eli-Berchoer L, Wapinski I, Friedman N, Kodesh E. Stress-related genomic responses during the course of heat acclimation and its association with ischemic-reperfusion cross-tolerance. *J Appl Physiol*. 2004;97:1496-1507.

Submitted: 25 January, 2008
Accepted after reviews: 25 December, 2008

doi: 10.2478/v10042-009-0017-z