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Kariometrical Studies on the Effect of Sex Hormones on the Adrenal Cortex in Rats Acclimated to Different Environmental Temperatures

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The effect of testectomy and sex hormones treatment on the nuclear volume and area in adrenocorticocytes of adult male rats, acclimated to different ambient temperatures (20±2°C and 35±1°C) was examined. The obtained results indicated that testectomy provoked increase of the nuclear volume and area in all adrenocorticocytes, regardless of the previous thermal acclimation, but it was statistically significant only for nuclei of zona reticularis'cells in rats from room temperature. The effect of testosterone depended on the previous thermal acclimation. Namely, testosterone didn't provoke significant changes of the examined parameters in animals from room temperature, but in heat acclimated rats the significant increase in these parameters was noted. The estradiol treatment, in both environmental temperatures, provoked statistically significant increase in nuclear parameters in all zones, which was more expressed in animals from room temperature.

Key words: kariometry, adrenocorticocytes, sex hormones, hyperthermia, rat.

Introduction

Numerous kariometrical studies on the endocrine glands, especially those concerning the adrenal gland, show that the nuclear volume of adrenocorticocytes represents a very important sign of their activity [11]. Many of them have also shown that estradiol provokes increase and testosterone decrease of cell and nuclear volumes in adrenal cortex, as well as volumes of mitochondria and SER — two organelles involved in steroid production [6].

The acute exposure of rats to a high environmental temperature provokes an increase of the nuclear volume of adrenocorticocytes, which is associated with the increased activity of the gland [12]. On the other hand, the chronical exposure of rats on elevated ambient temperature provokes decrease of nuclear volume in adrenocortical cells of the inner zones, which is correlated with the decreased activity of the gland [10].

Regarding the fact that there are no data about the effect of testectomy and sex hormones on the kariometrical parameters in adrenocorticocytes under elevated

ambient temperature, the aim of this study was to find the effects of sex hormones on the nuclear volume and area in heat acclimated rats, compared the same at room temperature.

Material and Methods

The experiments were performed on adult male laboratory rats, Wister strain, weighing 203-309 g. The access to food and water was ad libitum. The animals were divided into two groups: heat-acclimated group kept 30 days at 35±1°C and the control group kept at 20±2°C for several weeks. Each group was divided into 4 subgroups: 1) Control; 2) testectomized; 3) testectomized + testosterone; 4) testectomized + estradiol.

Animals were subjected to bilateral testestomy and 14 days after the operation they were hormonally treated. The hormonal application (testosteron propionate—(+)-17β-propionil-oksi-4-androsten-3-ona, and estradiol dipropionate—(+)-3,17-β-dipropionil-oksi-2,3,5-estratriena, "Galenika"-Belgrade) on testectomized rats was performed i.m. in doses of 1mg/100g b.w./daily for last 4 days. The animals were sacrificed 24 hour following the last dose. The adrenal glands were fixed in Bouin's solution, embedded in paraffin and cut at 3-4 μm. Paraffin sections were stained with hematoxylin and eosin. For kariometrical analyses specific software for digitalization and analysis "Lucia G" (Nikon) was used. The nuclear volume and area of 150 spherically nuclei from every zone of the cortex were measured. The nuclear area was determined automatically, but the nuclear volume was measured according to the formula:

$V=\pi/6*EqDia^3$.

EqDia is a mean diameter of the circle, which has same area as the measured nuclei.

Results were statistically evaluated by the Student's t-test.

Results

The results from our investigation were given as graphs illustrating the mean volume and area of nuclei in zona glomerulosa (Table 1), zona fasciculata (Table 2) and zona reticularis (Table 3) cells.

T a b l e 1. Mean value of nuclear volume and area in zona glomerulosa cells in rats from both ambient temperatures.

Nuclear Parameters	20±2°C				35±1°C			
	Intact (control)	test.	test.+T	test.+E	control	test.	test.+T	test.+E
Nuclear volume (μm^3)	94.27 ±2.04	95.26 ±2.54	92.11 ±2.70	106.68± 2.13^{c,B}	78.92 ±3.81	81.84 ±1.12	88.50 ±0.58^{a,A}	96.75 ±2.60^{c,C}
Nuclear area (μm^2)	24.98 ±0.37	25.17 ±0.43	24.58 ±0.48	27.14 ±0.37^{c,B}	22.17 ±0.72	22.74 ±0.21	23.96 ±0.11^{a,A}	25.41 ±0.45^{c,C}

Notes. Cont. (control); test. (testectomized); test+T (testectomized and treated with testosterone); test+E (testectomized and treated with estradiol). Values are given as mean±stand. dev. Significance of the values is given with the superscripts, compared with the control (a: $p<0.05$; b: $p<0.005$; c: $p<0.001$) and compared to the testectomized animals (A: $p<0.05$; B: $p<0.01$; C: $p<0.005$)

T a b l e 2. Mean value of nuclear volume and area in zona fasciculata cells in rats from both ambient temperatures: cont

Nuclear Parameters	20±2°C				35±1°C			
	Intact (control)	test.	test.+T	test.+E	control	test.	test.+T	test.+E
Nuclear volume (μm^3)	127.64 ±3.06	132.84 ±4.50	124.04 ±2.97	184.52± 2.88[b]	111.37 ±1.30	113.55 ±1.34	124.21 ±4.75[a]	150.84 ±2.49[b]
Nuclear area (μm^2)	30.59 ±0.49	31.41 ±0.71	30.01 ±0.48	39.12 ±0.40[b]	27.89 ±0.23	28.27 ±0.21	30.03 ±0.76[a]	34.18 ±0.37[b]

Notes. Cont. (control); test. (testectomized); test+T (testectomized and treated with testosterone); test+E (testectomized and treated with estradiol). Values are given as mean±stand. dev. Significance of the values is given with the superscripts, compared with the control as well as testecomized animals (a: $p<0.05$; b: $p<0.001$)

T a b l e 3. Mean value of nuclear volume and area in zona reticularis cells in rats from both ambient temperatures

Nuclear Parameters	20±2°C				35±1°C			
	Intact (control)	test.	test.+T	test.+E	control	test.	test.+T	test.+E
Nuclear volume (μm^3)	79.22 ±0.72	83.36 ±0.57[b]	79.96 ±5.51	108.32± 6.31[b,c]	74.32 ±5.08	73.70 ±1.41	88.55 ±5.84[a]	101.59 ±4.39[c]
Nuclear area (μm^2)	22.26 ±0.13	23.02 ±0.10[b]	22.34 ±1.06	27.41 ±1.08[b,c]	21.29 ±0.99	21.19 ±0.27	23.97 ±1.04[a]	26.25 ±0.77[c]

Notes. Cont. (control); test. (testectomized); test+T (testectomized and treated with testosterone); test+E (testectomized and treated with estradiol). Values are given as mean±stand. dev. Significance of the values is given with the superscripts, compared with the control as well as testectomized animals (a: $p<0.05$; b: $p<0.01$; c: $p<0.001$)

Two weeks after testectomy a slight increase in volume and area of cellular nuclei was observed in all the zones, regardless of the previous thermal acclimation. Nevertheless, the increase of these parameters was significant only for zona reticularis' cells (Table 1, 2 and 3).

In testectomized rats from room temperature, testosterone provoked a slight decrease in measured parameters compared to the testectomized animals. In heat-acclimated testectomized and testosterone treated rats a significant increase ($p<0.05$) of nuclear parameters can be observed (Table 1, 2 and 3).

The introduction of estradiol into testectomized animals from both ambient temperatures, showed a remarkable increase in volume and area of cellular nuclei in all zones (Table 1, 2 and 3), although these parameters in heat-acclimated rats were considerably smaller than those from room temperature.

Discussion

Testectomy provokes increase of cellular, nuclear and mitochondria volumes, SER proliferation, as well as activity of enzymes involved in steroid production [6,11]. The significant increase in nuclear volume and area of zona reticularis cells in testectomized rats from room temperature, obtained in our study, corroborates the data that testectomy affects mainly zona reticularis, which partly undertakes the hormonal function of testes [4,5,7]. Similarly, [15] found an increased activity of zona reticularis during first 4 weeks after testectomy, while substitution therapy with testosterone preserves further increase or brings them back to normal levels, due to the inhibitory effect of testosterone to HPA axis [6].

Biochemical studies [16] have shown that the adrenal glands of testectomized, heat-acclimated rats had an increased activity. This finding doesn't correspond with our results of a slight (not significant) increase of measured nuclear parameters. We presume that the period of 14 days after the testectomy (used in our studies) is probably not long enough to show significant increase of the nuclear volume and area of adrenocortical cells, although we have found some other signs (supracortical nodules, decreased lipid content), which indicate an elevated stimulation.

It is well known that testosterone inhibits RNA synthesis [8], as well as volumes of nuclei and organelles involved in steroid production and the activity of their enzymes [6]. In our study, the administration of testosterone in testectomized rats from room temperature showed only numerical decrease of measured nuclear parameters, besides evidently enlarged lipid vacuolisation of the adrenocorticocytes, and probably longer period of application is needed to provoke nuclear changes. On the other hand, our results of the increase in nuclear parameters of cells in all the zones in heat-acclimated testectomized rats is opposite with the well-known effect of this hormone in animals at room temperature. On the other hand, we've found a large destruction of the histoarchitecture in the two inner zones in all heat-acclimated testectomized and treated with testosterone animals. Therefore, we presume that the increase of nuclear parameters in this experimental group is probably a result of a compensation for the great destruction of the cortex and therewith, steroid secretor cells in above-mentioned regions, but we have to do more researches on this issue.

The administration of estradiol in testectomized rats from both ambient temperatures provokes significant increase in nuclear parameters in cells of all the zones. This corroborates the data of other authors [4, 5, 6], who found that estradiol doesn't stimulate only HPA axis, but also has a direct effect on adrenocorticocytes, as well as stimulating effect on thyroid gland [2]. Nevertheless, the effect of estradiol was less expressed at elevated ambient temperature, due to a decreased activity of other glands: pituitary [9], thyroid [13, 14] and gonads [2, 9] in rats acclimated to moderately high ambient temperature.

Conclusion

From the obtained results can be concluded that testectomy has a stimulating effect on the activity of adrenal cells of zona reticularis in animals from room tem-

perature, but has no effect on nuclear volume and area of heat-acclimated animals. We think that this is probably due to a shorter period after testectomy for analysis the gland, compared to data obtained from other authors.

The effects of sex hormones depend on the previous thermal acclimation of the animals. Namely, testosterone treatment of testectomized rats from room temperature doesn't show any effect of nuclear parameters in adrenocortical cells, while in heat-acclimated testectomized rats, kariometrical parameters point to an increased adrenocortical activity.

Estradiol treatment in testectomized rats from both ambient temperatures provokes kariometrical changes that indicate a stimulation of adrenocorticocytes and therewith increased cortical activity. These changes are more pronounced in animals from room temperature.

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