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MORPHOFUNCTIONAL CHARACTERISTICS OF THE ADRENAL CORTEX IN TESETCTOMISED RATS ACCLIMATED ON DIFFERENT AMBIENT TEMPERATURE

Popovska-Percinic Florina[1], Miteva Nada[2], Jordanova Maja[2], Ilieski Vlatko[1], Pendovski Lazo[1], Blagoevska Katerina[1]

[1]Department of functional morphology,
Faculty of veterinary medicine, Skopje, Macedonia
[2]Institute for Biology,
Faculty for Natural Sciences and Mathematics, Skopje, Macedonia
e-mail: florinap@fvm.ukim.edu.mk

ABSTRACT

The activity of the adrenal gland is connected with the activity of other endocrine glands, such as the gonads. The experiments were conducted on testectomized adult, male rats, Wistar strain, acclimated on two ambient temperatures: room and moderately high temperature. Bilateral testectomy was performed on the rats of both temperatures and analyses were made 15-20 days after surgery. It was shown that the adrenal weight of testec-tomized heat acclimated rats was signifi cantly increased. The widening of zona reticularis, shortening of zona fasciculata, as well as presence of supracortical nodules in the adrenal cortex of testectomized rats, regardless of the ambient temperature, was evidenced. Lipid content in the adrenocortical cells was lowered in both groups, but that was more prominent in testectomized rats from room temperature. The morphometric measurements of the nuclear area and volume were signifi cantly elevated only in zona reticularis cells of testectomised animals from room temperature. Those parameters were signifi cantly decreased in testectomized heat acclimated rats in comparison with the same values in the testectomized animals from room temperature. These results suggest that testectomy provokes changes towards increased activity of the adrenal cortex in both ambient temperatures, but those changes are less intense in heat acclimated animals.

Key words: testectomy, adrenal cortex, morphometry, heat acclimation

INTRODUCTION

The two main axes involved in the reaction of the organisms to different stressors are hypothalamo-hypophysis-adrenal axis (HHA) and simatico-adrenomedulary (SAM) axis. Adrenal gland plays an important role in both of them, and noticeable changes can be seen not only in its medulla, but also into its cortex (1,2). Certain gonadal changes are also determined in stress conditions, indicating mutual functional dependency of these two endocrine glands (1,2,3,4,5). These findings are confirmed by detection of specific sex hormone receptors, located in the nuclei of the adrenocorticocytes. This explains the large and diverse impact of gonadectomy

and sex hormones on the adrenal cortex (6). It is generally accepted that gonadectomy causes certain changes in the adrenal gland, its weight, cytoarchitecture and lipid content in the adrenocortical cells (7,8,9).

There are few data regarding the effect of the elevated ambient temperature upon the morphology of the adrenal cortex. Most of them are investigating the effect of the acute (short-term) exposure to high temperatures (10,11,12) and only some of them the effects of long-term exposure to elevated ambient temperature (12,13). On the other hand, there are even more insufficient data about the effect of testectomy on the adrenal cortex in rats previously acclimated to high ambient temperature and these studies are treating the issue from the physiological and biochemical point of view (13,14).

Regarding the fact that we couldn't find any paper about the effect of testectomy on the adrenal cortex in rats acclimated to high ambient temperature, the aim of this study was to investigate the changes caused by testectomy in cytomorphology of the adrenal cortex of rats acclimated to room temperature (20±2°C) and elevated ambient temperature (35±1°C).

MATHERIALS AND METHODS

The experiments were conducted on adult (3-5 months old) male laboratory rats, Wister strain, weighing 203-309g. The access to food and water was ad libitum throughout the entire experiment. The animals were divided into four groups: group I: intact rats (control group from room temperature); group II: testectomized rats at room temperature; group III: rats acclimated 30 days to 35±1°C (control group at high temperature) and group IV: testectomized rats acclimated 30 days to 35±1°C.

The acclimation of rats to high ambient temperature was made in hot chamber with controlled temperature of 35±1°C and relative humidity 35-45%. The animals were continually exposed 30 days to this temperature, before the testectomy was made. Bilateral testectomy was performed on the rats of both ambient temperatures and the analyses were made 15-20 days after the surgery.

For histological analyses, after the sacrifice of the animals, adrenal glands were put in the Bouin's fixative, embedded into paraffin and serially cut at 4-5µm. Paraffin sections were stained with hematoxylin-eosin and cresyl violet after Nissl. For morphometric analyses a specific computerized system for digitalizing and analyzing "Lucia G" (Nikon) was used.

The results were statistically processed by Student's t-test.

RESULTS

The mean values of the absolute and the relative adrenal weight were shown in Table 1, while the mean values of the nuclear area and volume of the adrenocoricocytes are shown in Table 2.

Table 1. Mean values of the body weight, absolute and relative adrenal mass

	20±2°C		35±1°C	
	intact (control)	testectomized	control	testectomized
Body weight (g)	268.02±12.74	277.71±6.33	219.76±6.51	220.71±7.81
Absolute weight (g)	33.33±1.1	36.37±6.11	25.86±0.48	32.41±1.03[a]
Relative weight (mg%)	12.72±0.91	12.98±1.88	11.93±0.37	14.83±1.06[a]

The values are the means ± S.D.
Statistical significance in correlation to control : a: $p<0.001$

Table 2. Mean values of the nuclear volume and area of the adrenocoricocytes

		20±2°C		35±1°C	
		intact	testectomized	control	testectomized
Nuclear volume (μm^3)	z.glomerulosa	94.27±2.04	95.26±2.54	79.92±3.81[b]	81.84±1.12
	z. fasciculata	127.64±3.06	132.84±4.50	111.37±1.30[c]	113.55±1.34
	z. reticularis	79.22±0.72	83.36±0.57[a]	74.32±5.08	73.70±1.41
Nuclear area (μm^2)	z.glomerulosa	24.98±0.37	25.17±0.43	22.17±0.72[b]	22.74±0.21
	z. fasciculata	30.59±0.49	31.41±0.71	27.89±0.23[c]	28.27±0.21
	z. reticularis	22.26±0.13	23.02±0.10[a]	21.29±0.99	21.19±0.27

The values are the means ± S.D.
Statistical significance in correlation to intact animals
a: $p<0.01$, b: $p<0.005$, c: $p<0.001$

Group I: intact rats (control group)

The adrenal cortex of the intact rats shows normal histological picture of zona glomerulosa, zona fasciculata (Fig. 1a) and zona reticularis (Fig. 1b).

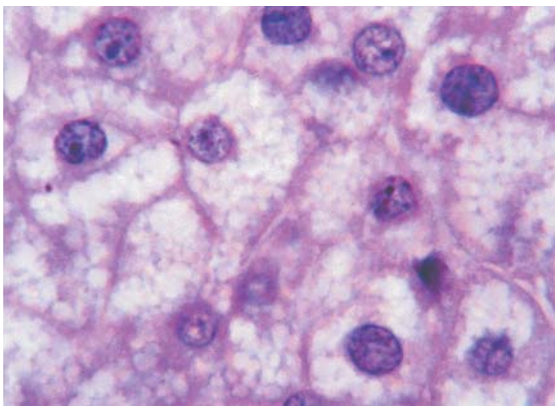

Figure 1a. Zona fasciculata in intact rats (control group)

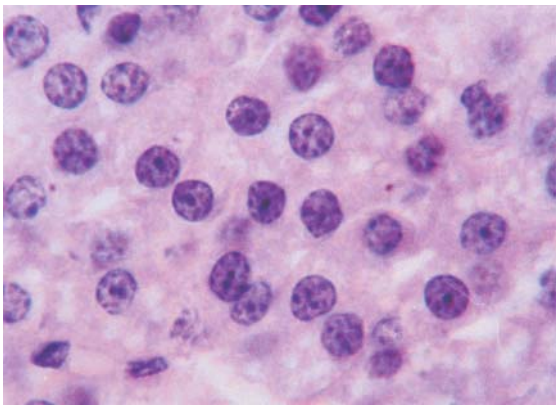

Figure 1b. Zona reticularis in intact rats (control group)

Group II: testectomized rats at room temperature

The analysis of the adrenal cortex of the rats in this group shows considerable changes in his histoarchitecture compared to the control group. Namely, the appearance of subcapsular nodules in the gland's surface is highly evident. These nodules are clearly divided from zona glomerulosa and composed of two types of cells (Fig. 2). In the central region of the nodules, the cells are larger with rich cytoplasm and euchromatic nuclei, while of the nodule's surface they are smaller, closely aggregated, with small heterochromatic nuclei. The width of the cortex is enlarged, but the width of the zona fasciculata visually seems to be narrowing, while zona reticularis is wither compared to the same in control group. The lipid droplets are evidently decreased in adrenocorticocytes of zona fasciculata (Fig. 3a) and reticularis (Fig. 3b). Morphometrical measurements showed slight increase in volume and area of cellular nuclei in all zones, but it was statistically significant only for zona reticularis cells ($p<0.01$). The absolute and relative adrenal weight show no significant changes compared to the control (Tab. 1 and 2).

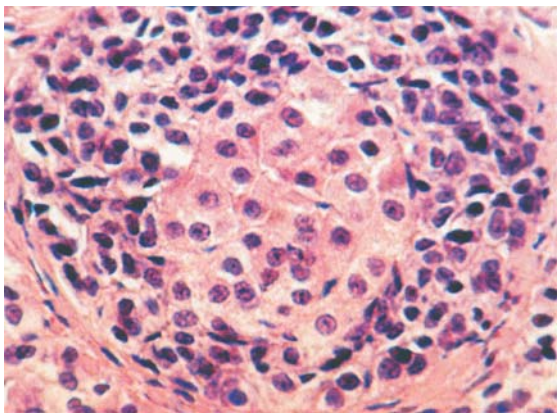

Figure 2. Adrenocortical proliferate as a nodulus on the gland's surface, H&E,x400

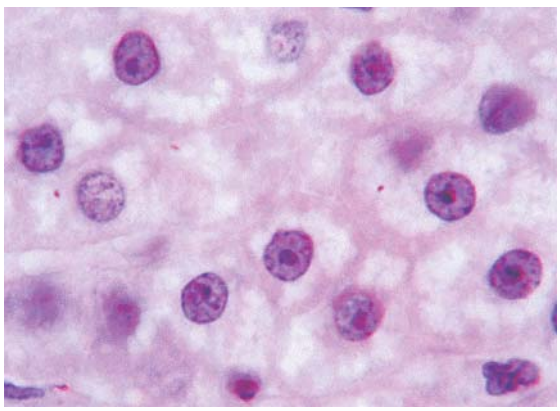

Figure 3a. Lower content of lipid vacuoles in the cytoplasm of zona fasciculata cells, H&E,1000x;

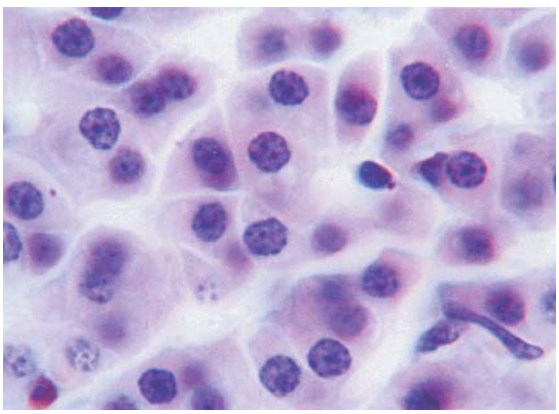

Figure 3b. Lower content of lipid vacuoles in the cytoplasm of zona reticularis cells, H&E,400x;

Group III: heat acclimated rats 30 days on 35±1°C (control group for heat)

The adrenal cortex has relatively well developed zona glomerulosa, spreading along the whole gland. The cells are with oval euchromatic nuclei. In the outer parts of fascicular zone, there are some regions composed of dark cells with smaller nuclei (Fig. 4). The increased vascularization of this region is evidenced as well. The amount of the lipid droplets has been decreased in the inner zones of the cortex, compared to the same in intact animals (Fig. 4 and 5). Zona reticularis's anastomozing cordons of cells have been closely gathered so that the sinusoidal spaces are barely noticed. Morphometric measurements showed that the absolute and relative adrenal mass, although numerically decreased, show no significant changes (Tab.1), while a significant decrease of nuclear volume and area in zona glomerulosa ($p<0.005$) and zona fasciculata cells ($p<0.001$) is evidented compared to the intact animals (Tab. 2).

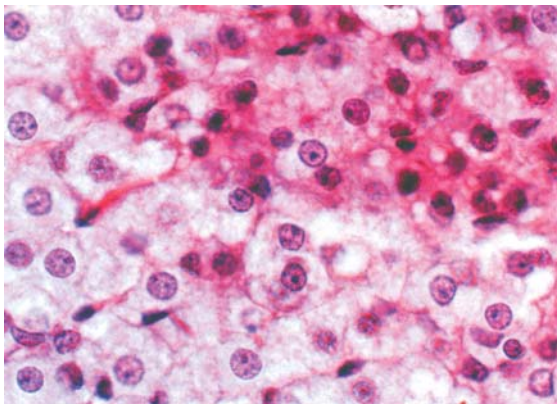

Figure. 4. Dark cells in zona fasciculata as well as lower lipid content in this zone, H&E, 600x

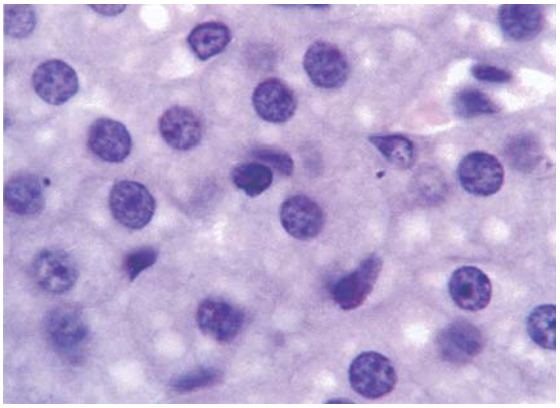

Figure 5. Lower lipid content in zona reticularis cells, H&E, 600x

Group IV: heat acclimated testectomized rats 30 days on 35±1ºC

The histological aspect of zona glomerulosa in this group shows similarities to same zone in heat acclimated rats. The presence of subcapsular nodules in the adrenal cortex is evidenced and is similar as in testectomized rats from room temperature (Fig. 6). In the fascicular zone the presence of cells undergoing degeneration can be noticed as well as the lipid content in the spongiocytes seen as small-dispersed vacuoles throughout the cytoplasm (Fig. 7). There is an evident hyperemia in the both inner zones. Zona reticularis is well developed.

The absolute and relative adrenal mass show significant increase ($p<0.01$), compared to the same in heat acclimated (control group) of rats (Tab.1). The nuclear volume and area of adrenocorticocytes show slight, but not significant increase, compared to control group at elevated ambient temperature (Tab.2).

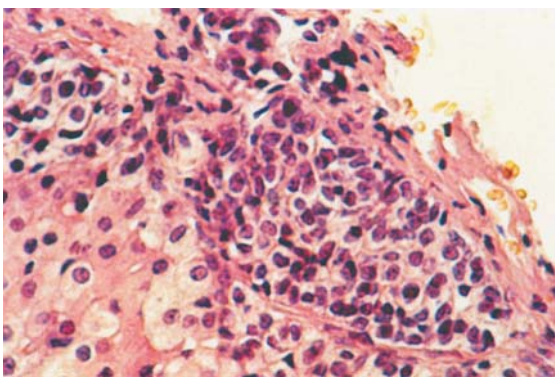

Figure 6. Subcapsular nodulus, H&E, 400x

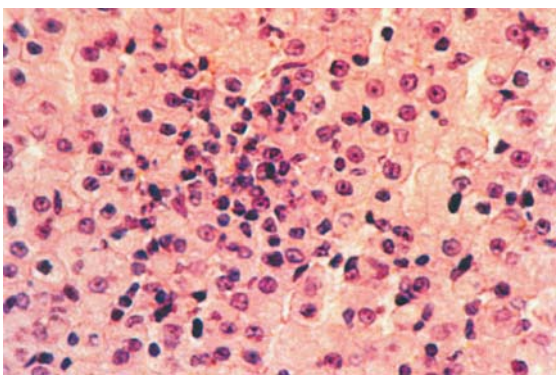

Figure 7. Degenerated cells and decreased lipid content in zona fasciculata H&E, 600x

DISCUSSION

In testectomized rats from room temperature the adrenal weight, although slightly elevated, showed no significant changes. These findings corroborate with the studies of Nicola (15), who registered elevation of the adrenal weight after a month from the orchiectomy, but not before. Other studies (5,7) have also evidenced initial elevation of the adrenal weight 2 months after testectomy, reaching the maximum after 3 months, after which started to decrease till normal (control) values. According to the previous authors, the effect of testectomy on the adrenal weight depends upon the time elapsed

from the operation. Therefore, we assume that, in our study, it is too soon for any significant changes in this parameter, due to the analyses that were made 15 days after testectomy. On the other hand, the adrenal weight of testectomized heat acclimated rats was significantly increased, which may be due to larger vascularization of the cortex in these animals found in our study. Prominent hyperemia is evidenced in the experiments of acute exposure (10) as well as chronic exposure on elevated ambient temperature (12). According to Hinson (16), elevated blood flow stimulates steroidogenesis, even when there are no other stimulators at all. Our studies are in coordinance with the results from others, who have also found no changes in adrenal weight after acclimation on moderate high ambient temperature (17).

In testectomized rats from room temperature, the hypertrophy of the adrenal cortex, due to the withering of the zona reticularis was evidenced, pointing towards elevated activity of the reticular zone, probably as a mechanism for compensation the lack of sex hormones. Increased steroidogenic capacity as well as function of the adrenocorticocytes from the inner zones of the cortex are registered in testectomized rats from room temperature (8,18). Withering of the two inner zones after testectomy has been evidenced by other authors and according to them is connected with the increased stimulation of these glands through increased secretion of ACTH (3,4,5,20). This is probably due to the fact that this gland and particular zona reticularis are involved in production of sex hormones (19). Some authors have found increased activation of the HPA axis after testectomy, caused by pituitary hypersensitivity to hypothalamus stimuli (20), elevated number (27) and area (7) of ACTH-immunoreactive pituitary cells.

The increased values for nuclear volume and area of zona reticularis cells in testectomized rats from room temperature, in our study, demonstrate increased cell activity, which corresponds to the results from other authors (8). After testectomy, an increased steroidogenic activity of the zona fasciculata and reticularis cells, shown by elevated mitochondria's volume and proliferation of the sER (smooth endoplasmic reticulum) key organelles for steroidogenesis is evidenced (5,8), as well as elevated enzymatic activity of cytochrome P450scc and 11β hydroxilase, resulting in increase of total steroidogenic production (20). Very few studies on the heat acclimated testectomized animals suggest elevated activity of the adrenal cortex expressed by increased concentration of plasma corticosterone (14). Regarding zona glomerulosa, there were no evident changes of the nuclear volume and area of the cells. This corresponds to the data obtained from others (5,7), according to which there are no changes neither in histological nor in ultrastructural level in the cells of this zone. According to these authors, this suggests the assumption that testectomy has no effect in this zone. That is probably true, due to the fact that this zone is not involved in the sex hormone synthesis (19).

We have noticed that the lipid content in the cytoplasm of adrenocorticocytes in testectomized animals from both ambient temperatures was evidently decreased, while in some parts of the cortex droplets were even absent, especially in rats from room temperature. These findings corroborate the results of other authors. They notices that testectomy provokes decreasing of the lipid content in the cells of zona fasciculata (7,21) and zona reticularis (8) and that decreased lipid content is correlated with the increased steroidogenic activity of the adrenocortical cells (9).

The appearance of the cortical nodules (islets) was evidenced in the testectomized rats from both environmental temperatures. Some authors consider them as indicators of a localized adrenocortical hyperplasia (12) and participate in steroid production, supported by markedly increased level of plasma ACTH (25). The hyperplastic changes morphologically are similar to histological changes of the adrenal cortex in people after prolonged application of ACTH and they are connected with the increased adrenal stimulation (26). We assume that the reduced incidence (occurrence) of these nodules in heat acclimated rats, registered in our study, is probably as a result of a suppressed activity of adrenal cortex during chronic exposure to moderately high ambient temperature (13).

We have found that all morphological parameters (increased adrenal weight, hyperemia, decreased lipid content and the appearance of subcapsular, mainly supracortcal nodules) in testectomized rats, regardless of the previous thermal acclimation indicate an increased adrenocortical function. Nevertheless, all these parameters are more pronounced at room temperature. We suppose that the reason of that is the chronic exposure to elevated temperature,

who has a suppressor effect on the adrenal cortex activity, mainly because of the drop of the ACTH plasma concentration (13), decreased activity of pituitary gland (22), thyroid gland (22,23,24) as well as gonads (12,13) in animals after chronic exposure to high ambient temperature.

CONCLUSION

We can conclude that, regardless of the ambient temperature, testectomy has a stimulating effect on the cytomorphology of the adrenal cortex, which is less pronounced in heat acclimated animals. The reason of that is, probably, long term acclimation (chronic acclimation), which reduces the changes aroused from testectomy.

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