Chronic Exposure to Constant Light Affects Morphology and Secretion of Adrenal Zona Fasciculata Cells in Female Rats

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Abstract. The effect of chronic exposure to light of adult Wistar rats on growth and function of adrenal *zona glomerulosa* (ZG) and *zona fasciculata* (ZF) were examined. The females were exposed to continuous light of 600 lux for 95 days, starting on day 30 of age. The controls were kept under a 12 : 12 h light-dark cycle, at ambient temperature. The rats were sacrificed by decapitation and the left adrenal gland of each animal was dissected out and prepared for morphometric analyses. In animals exposed to chronic lighting, the absolute and relative volume of ZG were insignificantly increased by 5 % ($p > 0.05$) compared to controls. The volume of ZG cells and their nuclei were insignificantly changed by 1 % ($p > 0.05$) in comparison with corresponding controls. The absolute and relative volume of ZF were significantly increased (by 14 and 9 %, respectively; $p < 0.05$), as compared to controls. The volume of ZF cells and their nuclei were significantly increased (by 12 and 9 %, respectively; $p < 0.05$). Serum concentration of corticosterone was also significantly ($p < 0.05$) increased by 13 % in light-exposed group in comparison with control rats. These findings suggest that continuous exposure of female rats to constant light increased growth and secretory activity of ZF cells.

Key words: *Zona glomerulosa* — *Zona fasciculata* — Corticosterone — Female rats — Constant light

Introduction

In photoperiodic mammals such as rats, light is an important regulator of secretion of several pituitary hormones (Freeman et al. 2000). Changes in the photoperiod

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causes various disturbance in the function of the female rat reproductive system (Blake 1976; Ivanišević-Milovanović et al. 1995). Chronic exposure of adult female rats to constant light, presumably acts *via* activating the stress system, i.e. the paraventriculo-infundibular corticotropin-releasing hormone (CRH) system (Liposits et al. 1984). Same studies have suggested that vasopressin (AVP) may be necessaary for proper functioning of CRH neurons that regulate adrenocorticotropin hormone (ACTH) synthesis and secretion (Al-Damluji 1988). Stress-induced responses of neuroendocrine effector systems as well as central catecholaminergic systems appear to be stressor-specific and to have distinct central and peripheral pathways and mechanisms (Pacak et al. 1995). The following pituitary cell types were reported to be involved in stress reaction: i) the cells synthesizing proopiomelanocortin, a large precursor protein for ACTH, melanostimulating hormone and some opioids, and ii) the cells producing prolactin (PRL) and growth hormone (Molitch 1997). The pituitary ACTH cells are under the influence of paraventriculo-infundibular CRH producing neurons, known as the most stress-sensitive neurons in the brain (Pantić 1995). Gender differences in brain structures may be responsible for sexually dimorphic stress responses (Traustadottir et al. 2003). Rivier (1999) reported that the pituitary response to corticotropin-releasing factor was larger in intact females compared to intact males.

ACTH cells are the first differentiated pituitary hormone-producing cell type. These cells control adrenal growth and development, as well as steroidogenic maturation of fetal adrenal glands. During ontogenesis in rat hypophysis, the first immunopositive ACTH cells appear on fetal day 16 in the *pars distalis* and one day later, in the *pars intermedia* (Chatelain et al. 1974). ACTH enters the systemic blood and stimulates the adrenal glands to secrete glucocorticoid hormones – corticosterone in rats and cortisol in humans (Nankova et al. 1996). On the other hand, the action of glucocorticoid hormones is directed toward the modulation of energy metabolism and immune system, allowing an organism an adaptive response to stress (Ehrhart-Bornstein et al. 1996), that serve to maintain homeostasis and survival.

Our interest in this paper was focused on the morphofunctional characteristics of *zona glomerulosa* (ZG) and *zona fasciculata* (ZF) cells in adrenal cortex after prolonged exposure to constant light in adult female rats.

Materials and Methods

Adult female rats of Wistar strain (210–230 g), bred at the Institute for Biological Research in Belgrade were used. One group of animals was exposed to continuous light of 600 lux for 95 days, starting on day 30 of age. The control age-matched rats were maintained under a 12 : 12 h light-dark cycle, at ambient temperature. Food for laboratory rats (produced by D. D. Veterinarski zavod, Subotica, Serbia and Montenegro) and drinking water were available *ad libitum*. Experimental protocols were approved by the Local Animal Care Committee and conformed to the

recommendations given in "Guide for the Care and Use of Laboratory Animals" (1996, National Academy Press, Washington D. C.).

Protocol and light microscopy

After 95 days, two groups of rats (control and chronically exposed to light) were anesthetized with ether and decapitated. Blood was collected for later corticosterone level determination. Left adrenal glands were excised, fixed in Bouin's solution, embedded in paraffin and serially cut into 5 μm thick sections which were stained with hematoxilin-eosin and examined under a light microscope (Opton).

Morphometry

Stage 1: Zonation of the adrenal gland

In order to evaluate the volume densities of the adrenocortical zones, every tenth section of the gland was analyzed using a magnification of $\times$125 and the multipurpose test system M_{42} (Weibel and Gomez 1962). The absolute volume of the glands was calculated on the basis of their weight, assuming an average specific gravity of the adrenal of 1.039 g/cm^3 (Swinyard 1938).

Stage 2: Size and number of adrenocortical cells

The volume densities of nuclei and cytoplasm of parenchymal cells were estimated on a screen at $\times$1000 magnification, using again the same multipurpose test system M_{42}. For each adrenal gland, a single paraffin section containing medulla was chosen and 30 test areas of ZG and 50 test areas of ZF were analyzed. On the basis of earlier karyometric studies (Malendowicz 1974), the shape coefficient β was assumed to be 1.382 for ZF and 1.500 for ZG cells. It relates N_v (number of cells counted *per* unit volume) to N_a (number of cells counted *per* mm^2) and V_v (volume density), depends on the axial ratio of estimated nuclei. The number of adrenocortical cells nuclei *per* mm^3 was calculated according to the method of Weibel and Gomez (1962). Since the rat adrenocortical cells are mononucleated, the numerical density of nuclei corresponds to the number of cells *per* mm^3.

Digital images were made on a DM RB photomicroscope (Leica, Wetzlar, Germany) with a JVCTK 1280E Video Camera (Leica) for the acquisition and analysis of the images.

Biochemical analyses

Serum levels of corticosterone in control and chronic light-treated rats were measured by RIA method (^{125}I RIA Kit, Biochemicals, Costa Mesa, CA, USA).

Statistical analyses

Biochemical and morphometric data obtained from each rat were averaged *per* experimental group and standard deviation of the mean (S.D.) was calculated using Student's *t*-test. A probability value of 5 % or less was considered statistically significant.

Results

Body weight, absolute and relative weight of the adrenal gland

Body weight, absolute and relative weight of the adrenal gland were significantly decreased ($p < 0.05$) in animals exposed to constant light by 25, 39 and 32% respectively, compared with control females (Table 1).

Table 1. Effects of constant exposure to light on body weight and absolute and relative weights of adrenal gland in adult female rats

	Body weight (g)	Absolute adrenal weight (mg)	Relative adrenal weight (%)
Controls	311.0 ± 11.4	31.1 ± 2.2	10.7 ± 0.7
Constant light	231.6 ± 9.9* (−25%)	19.0 ± 2.8* (−39%)	7.3 ± 0.8* (−32%)

Values are means ± S.D. ($n = 5$); * $p < 0.05$ *vs.* control.

Adrenal cortex

All three cortical zones of the adrenal gland in all examined preparations were clearly visible: ZG, ZF and ZR (*zona reticularis*). The absolute and relative volumes of adrenal cortex in both groups of rats were insignificantly changed as seen from the Figs. 1 and 2.

Zona glomerulosa

The ZG is arranged in closely packed ovoid clusters. The cells of the ZG are relatively small and columnar or pyramidal. The nuclei are round or oval with an evidence nucleolus. The shape of ZG cells in animals chronically exposed to constant light was not significantly changed, but the cytoplasm in these cells is lighter compared to controls (Fig. 3A,B). The absolute and relative volume of ZG were insignificantly increased by 5%, ($p > 0.05$) in comparison with controls (Figs. 1 and 2). The volume of ZG cells and their nuclei were insignificantly changed by 1% ($p > 0.05$; Fig. 4A,B).

Zona fasciculata

The ZF is large with polyhedral cells. The cells are arranged in long straight cords, one or two cells thick, that are separated by sinusoidal capillaries. In control rats in this zone are seen both, light and dark stained cells (Fig. 3A). In rats chronically exposed to constant light in ZF, the longer cells with lighter cytoplasm are seen (Fig. 3B). The absolute and relative volume of this zone were significantly increased ($p < 0.05$) by 14 and 9% respectively, in comparison with control rats (Figs. 1 and 2). The volume of ZF cells and their nuclei were significantly increased

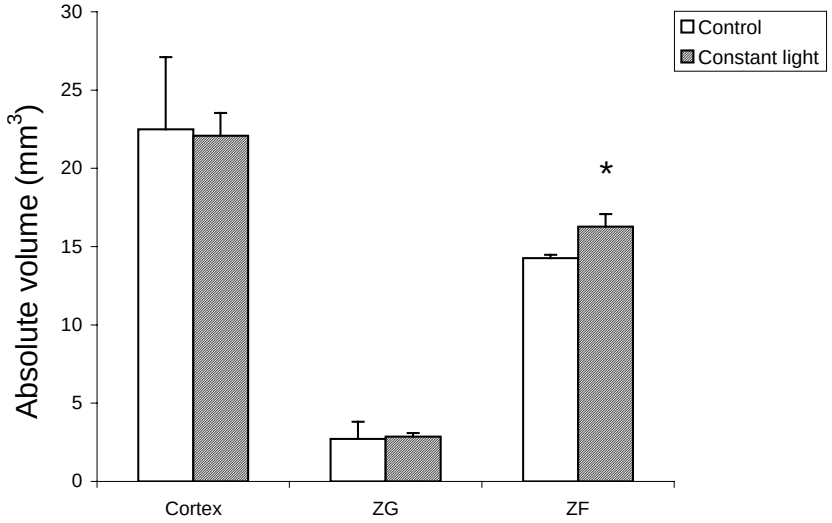

Figure 1. Absolute volumes (mm^3) of adrenal gland cortex, *zona glomerulosa* (ZG) and *zona fasciculata* (ZF) after chronic exposure to constant light. Values are means ± S.D. ($n = 5$); * $p < 0.05$.

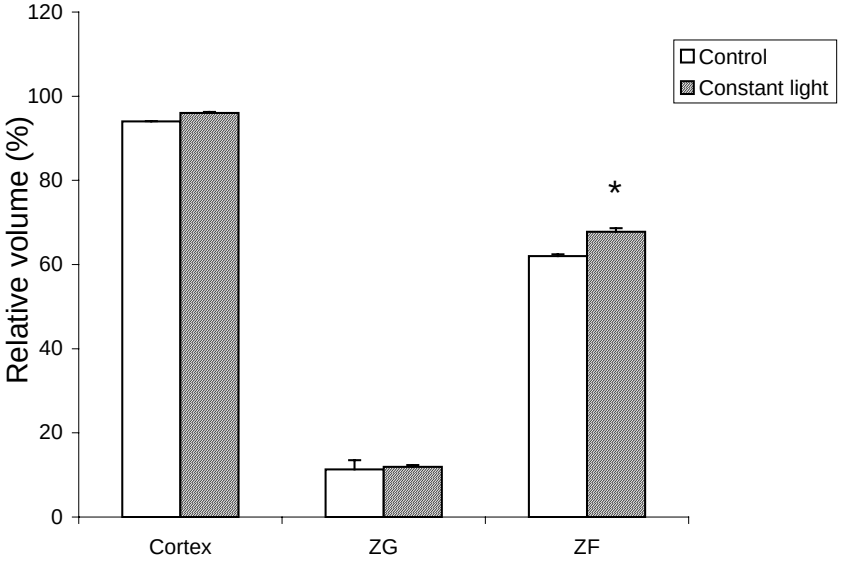

Figure 2. Relative volumes (%) of adrenal cortex, ZG and ZF after chronic exposure to constant light. Values are means ± S.D. ($n = 5$); * $p < 0.05$.

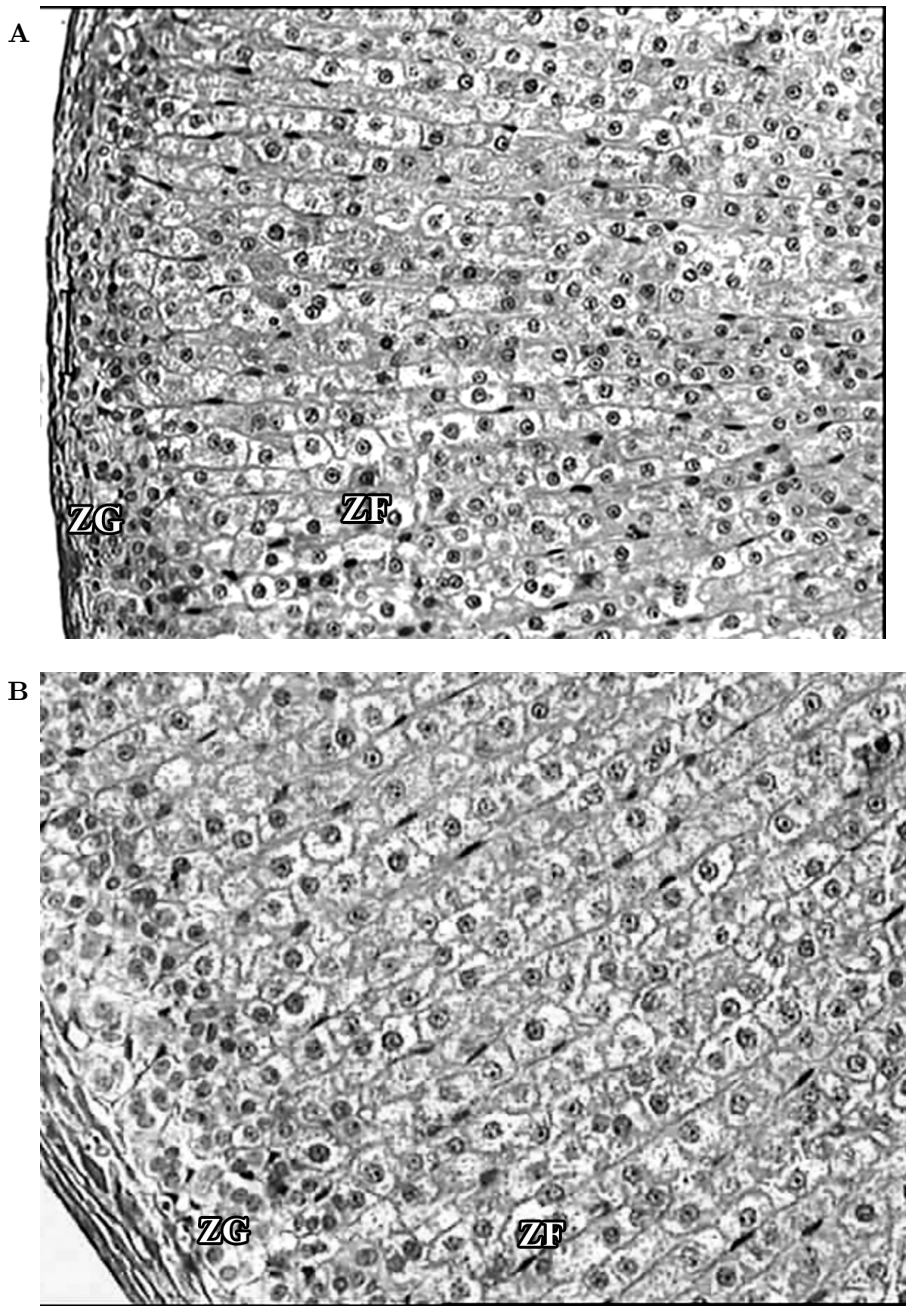

Figure 3. Histochemically labelled ZG and ZF cells in adrenal cortex of female rats in: A. control rats, B. after chronic exposure to constant light. (H&E, objective magnification ×20).

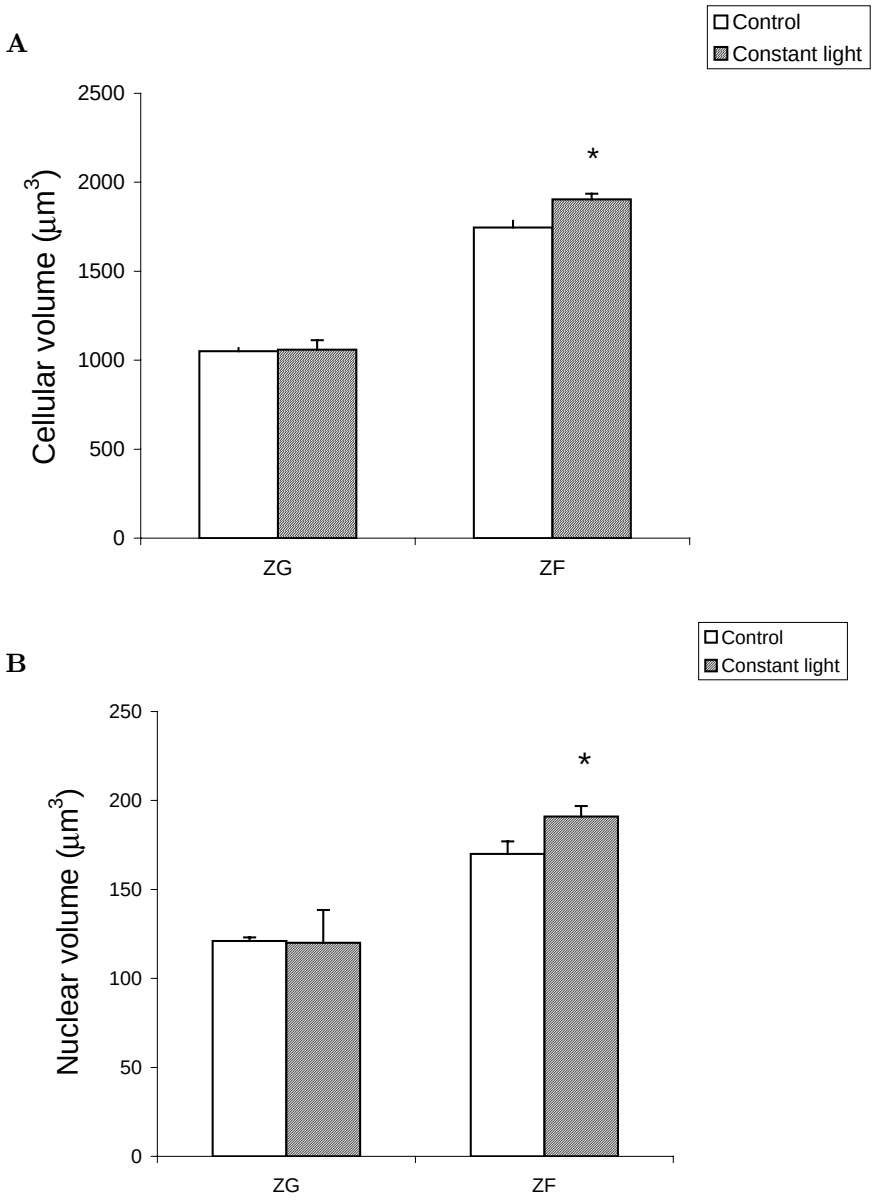

Figure 4. Panel A: cellular volume (μm^3) of ZG and ZF cells. Panel B: nuclear volume (μm^3) of ZG and ZF cells. All values are means $\pm$ S.D. ($n = 5$); * $p < 0.05$.

($p < 0.05$) by 9 %, and by 12 % respectively, in comparison with corresponding controls (Fig. 4A,B).

Serum concentration of corticosterone was significantly increased ($p < 0.05$)

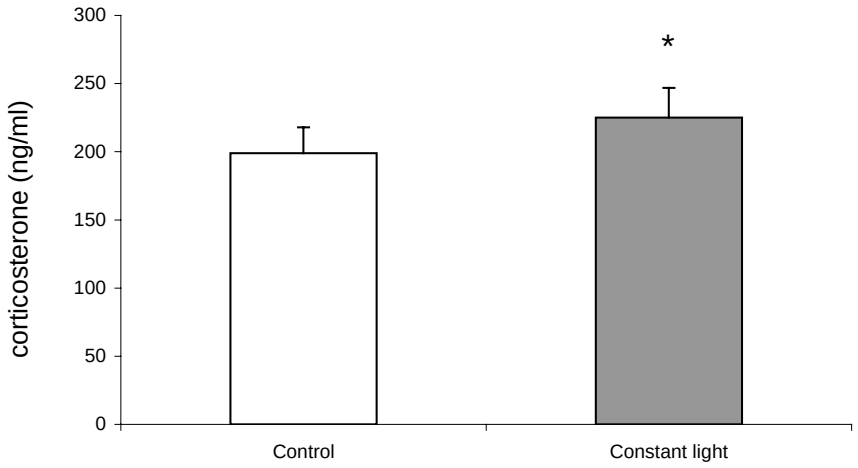

Figure 5. Serum concentration of corticosterone (ng/ml) in adult female rats. Data are expressed as mean values ± S.D. ($n = 5$); * $p < 0.05$ *vs.* control.

by 13 % in animals chronically exposed to light in comparison with control females (Fig. 5).

Discussion

The results obtained throughout the present study demonstrate that long-term exposure to constant light significantly decrease absolute and relative adrenal weight of adult female rats. These results are in accordance with Ivanišević-Milovanović et al. (1995) who reported that exposure to constant light for six weeks led to a significantly lower adrenal gland weight.

Our results showed that prolonged exposure to light of 600 lux, activated stress system and consequently the ZF cells became significantly hypertrophy and very light in colour, in comparison with female controls maintained under conditions of 12/12 h light-dark cycle. The level of corticosterone in blood was also significantly increased in the experimental group of rats in relation to control value. The hypertrophy of ZF cells and increase of corticosterone in serum may be explained as a consequence of increased activity of ACTH cells as we previously reported (Milošević et al. 2003).

Light is the most important regulatory factor for suprachiasmatic nucleus (SCN) activity, that is synchronizing the endogenous circadian rhythm with exogenous light-dark cycle (Rusak and Groos 1982). It was earlier observed that chronic exposure of adult female rats to constant light activates the stress system, namely, paraventriculo-infundibular CRH and AVP system (Liposits et al. 1984; Ferrini et al. 1997). The mechanisms responsible for the circadian release of CRH

and ACTH are still far from being fully understood, but it appears to be controlled by one or more pacemakers, including the SCN (Stratakis and Chrousos 1997). The SCN is the mammalian biological clock that generates the daily rhythms in physiology processes and behaviour (Scheer et al. 2001). Both ACTH and PRL cells play an important role in the regulation of behavioural adaptation of an organism to environmental changes. Data reported previously by Pantić (1995) showed a reciprocal interrelationship in the response of PRL and ACTH-corticosterone system to stress. In adult mammals, adrenal growth and steroidogenesis are regulated by ACTH (Lehoux et al. 1998), which acts through interaction with a specific melanocortin receptors specifically expressed in the adrenal cortex (Mountjoy et al. 1992). We have already reported (Milošević et al. 2003) that the growth and function of the ACTH cells are significantly increased (by 22 and 13 %, respectively) in female rats exposed to constant light for 95 days, compared to controls. The similar results were obtained in female rats exposed to constant light for six weeks (Ivanišević-Milovanović et al. 1995). Some studies are consistent in claiming that the neuroendocrine mediation of the stress response is very probably multifactorial (Gala 1990) and that the SCN outputs, important for neuroendocrine rhythms, are axonal rather than humoral (Mayer-Bernstain et al. 1999).

Some authors have reported that exposure of adult female rats to constant light led to development of polycystic ovaries, similar to that seen in human idiopathic polycystic ovarian disease (Mahajan 1988; Baldissera et al. 1991). In addition, it was shown that prolonged exposure of female rats to continuous light leads to an increased ACTH secretion, which is responsible for the appearance of ovarian endocrine dysfunction, perhaps through the ACTH effect on the adrenal cortex (Ivanišević-Milovanović et al. 1995).

On the basis of the results obtained throughout this study, it can be concluded that continuous exposure of adult female rats to constant light of 600 lux significantly stimulates function and growth of ZF cells.

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