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THE EFFECTS OF ESTRADIOL AND HUMAN CHORIONIC GONADOTROPIN ON ACTH CELLS IN PERIPUBERTAL FEMALE RATS: A HISTOLOGICAL AND STEREOLOGICAL STUDY

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Abstract - The effects of estradiol (EDP) and human chorionic gonadotropin (HCG) on histological and stereological features of adrenocorticotropic (ACTH) cells in peripubertal female rats were examined. The first group of females received five injections of EDP (0.25 mg/kg b.w.), every second day from the 4th to 14th day after birth, and was killed at the peripubertal stage. The second group of females was given two injections of pregnyl-gonadotrophinum chorionicum (HCG; 50 IU/kg body weight) on the 36th and 37th days after birth, and it was killed 24 h after the last treatment. The controls were injected with an equivalent volume of the corresponding vehicle. ACTH cells were immunohistochemically labeled and stereologically evaluated. Stereological analysis showed that the volume of ACTH cells and their volume density in peripubertal females treated with EDP, were decreased by 15.6% and 53.8% ($p<0.05$), respectively, compared to the controls. In HCG-treated animals, the observed parameters were increased by 39.2% and 15.4% ($p<0.05$), respectively, in comparison with the control females. These findings suggest that the application of EDP or HCG exerted opposite effects on the stereological features of pituitary ACTH cells.

Key words: Estradiol, HCG, ACTH cells, histology, stereology

INTRODUCTION

The pituitary *pars distalis* is a heterogeneous tissue comprised of different hormone-producing cells, most of which are influenced by estrogen (Sekulić et al., 2006). Certain amounts of estrogen are observed in the endocrine tissue of rats in the nuclei of all cell types, including acidophiles, basophiles and chromophobes (Pelletier, 2000). Friend et al. (1997) identified several estrogen mRNA isoforms in the rat pituitary gland, and examined their regulation by gonadal steroids. Estradiol acts *via* the nuclear estrogen receptor (ER) isoforms α and β, which serve as transcription factors after binding estrogens (Kuiper et al., 1997). In addition, it was shown that estrogen treatment decreases the level of POMC mRNA and lowers the ACTH response in stress-stimulated ovariectomized adult female rats (Redei et al., 1994). In our previous study, we reported that commercial estrogen decreased the immunohistomorphometric characteristics of rat ACTH cells in the juvenile period when applied during the neonatal period (Milošević et al., 2012).

Placental human chorionic gonadotropin (HCG) is composed of 244 amino acids. HCG is the essential hormone during pregnancy, maintaining the *corpus luteum* during early gestation. It is a heterodimeric

glycoprotein with two subunits. The α subunit is identical to the luteinizing (LH) follicle stimulating (FSH) and thyroid stimulating (TSH) hormones, The β subunit is responsible for hormone-specific biological functions (Canfield and Ross, 1976; Pierce and Parsons, 1981). HCG significantly increases the number of pituitary FSH, LH and TSH cells (Lovren et al., 1997; Sekulić et al., 2006). In animals that were treated with HCG during the neonatal period and killed during the juvenile period of life, ACTH cell and nuclear volumes were unchanged; however, the volume density was significantly decreased in comparison with the controls (Milošević et al., 2012).

The present study focused on the histological and stereological features of immunopositive ACTH-producing cells of female rats that were neonatally treated with multiple doses of estradiol or with two doses of HCG, and killed during the peripubertal period of life.

MATERIALS AND METHODS

All animal procedures complied with the EEC Directive (86/609/EEC) on the protection of animals used for experimental and other scientific purposes, and were approved by the Ethical Committee for the Use of Laboratory Animals of the Institute for Biological Research Siniša Stanković, University of Belgrade. Wistar female rats that were bred at the Institute for Biological Research "Siniša Stanković", Belgrade, Serbia, were used. The animals were kept under a 12 h light-dark cycle at 22±2 °C. They had free access to food (obtained from the Veterinarski zavod Subotica, Subotica, Serbia) and water. Females were divided into three groups of five animals each. The first group received five intraperitoneal (i.p.) injections of estradiol dipropionate (EDP; 0.25 mg/kg b.w. (ICN Galenika Pharmaceuticals, Belgrade, Serbia) every second day from the 4^{th} to 14^{th} day after birth. The rats were killed at the peripubertal stage (38^{th} day of life). The second group of females was given two i.p. injections of pregnyl-gonadotrophinum chorionicum (HCG, 50 IU/kg; N.V. Oregon, Netherlands) on the 36^{th} and 37^{th} days of life. These animals were killed 24 h after the last treatment. The peripubertal female controls were injected with an equivalent volume of the corresponding vehicle and killed at the times as the two previous groups.

Pituitary glands were excised, weighed, fixed in Bouin's solution for 48 h and embedded in paraplast. The relative pituitary weights were calculated from the ratio of the measured pituitary weight and the body weight for each animal. Serial 5-μm thick pituitary sections were mounted on gelatin-coated glass slides. ACTH-producing cells were identified by immunohistochemistry, using the peroxidase-antiperoxidase method as already described (Milošević et al., 2012). The stereological analysis was conducted using the M_{42} multipurpose test (Weibel, 1979), while the ACTH-producing cell and nuclear volumes (Vc, μm^3; Vn, μm^3, respectively), as well as their relative volume density (V_{VC}; %) were determined as described previously (Ajdžanović et al., 2009; Milošević et al., 2012). The stereological data obtained from each group were statistically evaluated by the Student's t-test. A probability value of 5% or less was considered statistically significant.

RESULTS AND DISCUSSION

Data for body weights, absolute and relative pituitary weights are summarized in Table 1. The body weight was significantly ($p<0.05$) decreased in EDP-treated peripubertal female rats by 20.6%, compared to the controls. A reduced body weight of rats treated with 17β-estradiol benzoate (Dubuc, 1974) or estradiol valerate (Köhler-Samouilidis et al., 1988), was previously. We have also demonstrated the reduction of body weights in juvenile female rats after the treatment with EDP during the neonatal period (Milošević et al., 2012). Gonadal steroids are important factors that influence food intake and body weight in mammals, however, the hormonal effects on these processes are particularly striking in female rats (Butera, 2010). Estradiol has been observed to inhibit feeding in animals, but the mechanism(s) mediating its effects are still not clear (Geary, 2001). In peripubertal female rats, HCG insignificantly ($p>0.05$) affected body weights (Table 1). It was earlier observed that the treatment of obesity with injections of HCG was

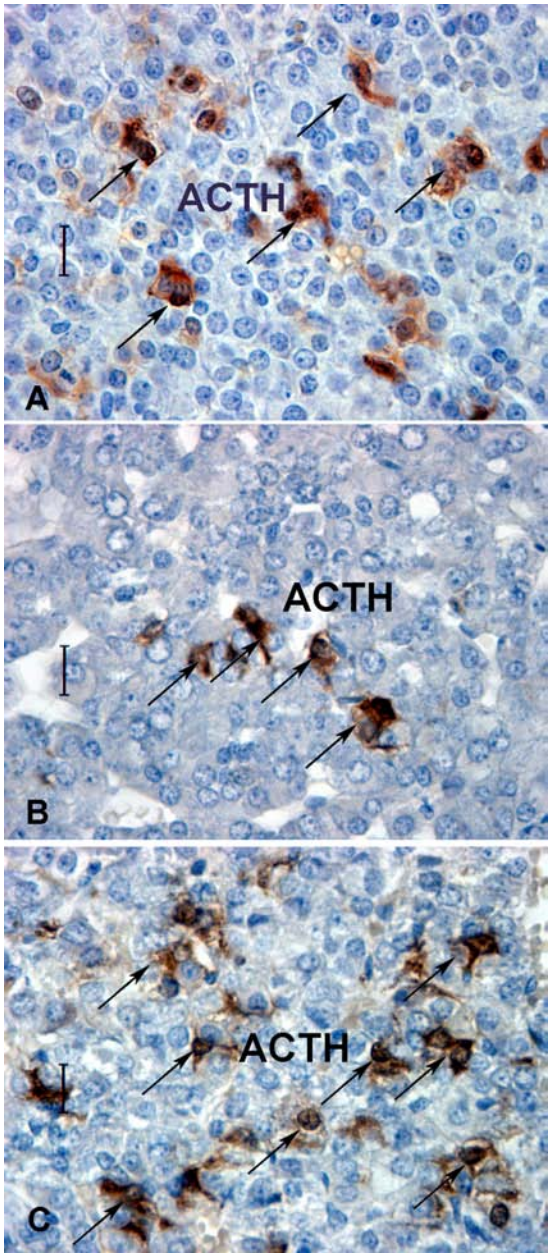

Fig. 1. Immunoreactive ACTH-producing cells in the *pars distalis* of the pituitary gland from control (1A), EDP - (1B) and HCG - treated animals (1C). (PAP, bar = 8 μm).

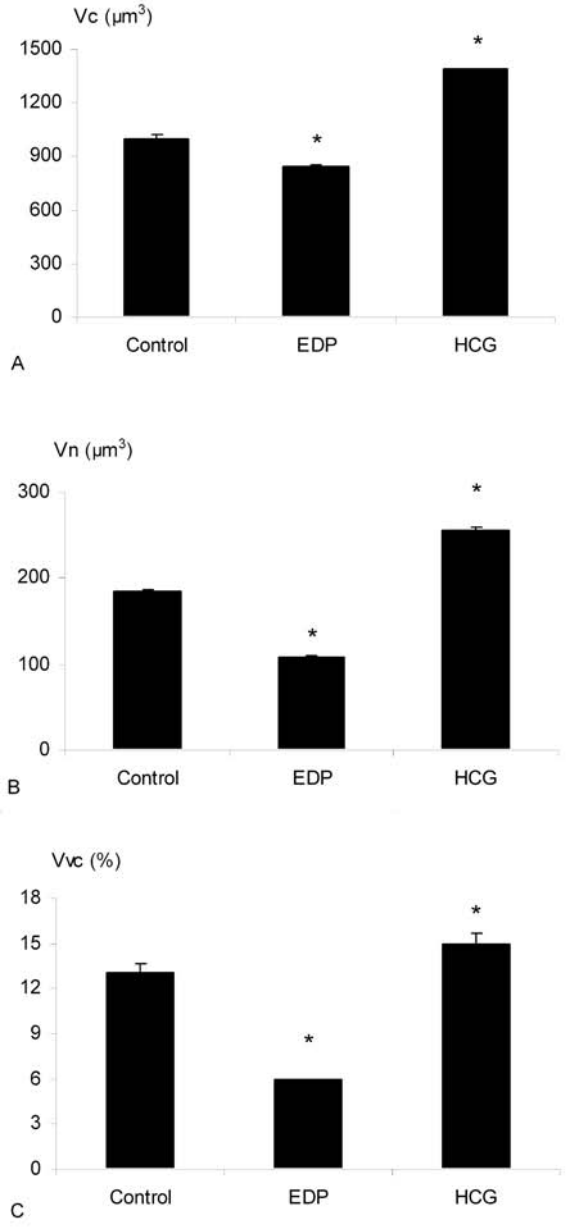

Fig. 2. Cell volume (Vc; μm³) of ACTH-producing cells **(A)**; nuclear volume (Vn; μm³) of ACTH-producing cells **(B)**; relative volume density (Vvc; %) of ACTH-producing cells expressed as a percentage of total gland tissue **(C)**. All values are means ± SD; n = 5 animals per group; *p<0.05 *vs.* controls.

without noticeable effects (Lijesen et al., 1995), which is in line with our results.

In EDP-treated peripubertal female rats, the absolute and relative pituitary weights were significantly (p<0.05) increased by 60.3% and 101.9%, respectively, in comparison with the controls (Table 1). The absolute and relative pituitary weights revealed a tendency for an increase in peripubertal and adult

Table 1. The effects of EDP and HCG on body weight, absolute and relative pituitary weights in peripubertal female rats.

Groups	Body weight (g)	Absolute pituitary weight (mg)	Relative pituitary weight (mg%)
Control	143.6±3.5	7.3±0.1	5.1±0.5
EDP	114.0±6.7* (-20.6%)	11.7±1.1* (+60.3%)	10.3±0.9* (+101.9%)
HCG	151.3±7.5 (+5.4%)	9.7±0.7* (+32.9%)	5.1±0.1 (0.0%)

Means ± SD, n=5; *p<0.05 *vs.* control rats

male rats after treatment with the estradiol valerate (Köhler-Samouilidis at al., 1998). In Sprague-Dawley rats, the absolute and relative pituitary weights were also increased after ethinylestradiol application (Kinomoto et al., 2000). This could be explained by an increased number of chromophobes (Pantić, 1995), PRL (Van Bael and Denef, 1996), as well as LH and FSH cells (Lovren et al., 1997; Medigović et al., 2012).

The ACTH-immunopositive cells in the control peripubertal females were mostly located in the central part of the pituitary *pars distalis*. They were often present as small groups in close proximity to numerous capillaries. In all groups, ACTH cells were irregularly shaped, often with expressed cytoplasmatic projections (Fig 1A-C). ACTH-immunopositivity was granular, uniformly distributed throughout the relatively small portion of cytoplasm surrounding the prominent nuclei (Fig 1A). In EDP-treated peripubertal animals, the ACTH-immunopositive cells were darker and smaller, although their location and shape remained as in the controls (Fig 1B). After HCG treatment, the shape of ACTH cells was not changed in comparison with the controls, but they become more numerous, with peripherally distributed granules (Fig 1C).

The stereological parameters described herein are presented in Fig. 2A-C. ACTH cell and nuclear volumes, as well as their volume densities in peripubertal female rats treated with EDP were significantly decreased by 15.6%, 40.8% and 53.8% (p<0.05), respectively, compared to the controls (Fig. 2A-C). An earlier study demonstrated that the neonatal period of life is critical for rat development and that administration of estrogens during this stage expresses a permanent effect on the neuroendocrine system (Pantić, 1995). We have recently shown that the neonatal treatment of female rats with EDP decreased the immunohistomorphometric parameters of ACTH cells in the juvenile period (Milošević et al., 2012). The mechanism of estradiol action is intriguing and most likely indirect, *via* somatostatin release (Milošević et al., 2012).

HCG treatment significantly (p<0.05) increased the absolute pituitary weight by 32.9% in comparison with the controls. In addition, the volume of ACTH cells and nuclei, as well as their volume density, after HCG treatment, were significantly increased by 39.2%, 39.1% and 15.4% (p<0.05), respectively, compared to the controls (Fig.2A-C). HCG is a heterodimeric glycoprotein, well recognized as the key player during pregnancy, while its amino acid sequence of the α subunit is identical to that of LH, FSH and TSH (Priece and Parsons, 1981). Our earlier studies reported that HCG significantly increases the number of FSH and LH cells (Lovren et al., 1997), as well as the volume of TSH cells (Sekulić et al., 2006) in rats. Adrenocortical steroidogenesis in healthy women proceeds without the significant role of LH/HCG (Piltonen et al., 2002), but it was observed that HCG can modulate this process *in vitro* when ACTH levels are low (O'Connell et al., 1994). A several-fold increase of serum corticosterone, with histological signs of adrenocortical stimulation, was observed in LH-overexpressing transgenic mice (Kero et al., 2000). Furthermore, in polycystic ovary syndrome, when LH levels are high, the adrenal cortex manifests

increased sensitivity/response to ACTH (Lachelin et al., 1979; Chang et al., 1982). Keeping in mind the structural similarity between HCG and LH, as well as the already mentioned LH-induced hypersensitivity of the adrenal cortex to ACTH, it remains possible that ACTH cells arrest their secretion *via* negative feedback, which resulted in an increase of their stereological parameters in our study.

Based on the results presented here, it can be concluded that the application of EDP or HCG induced significant, but opposite, changes on the histological and stereological features of the pituitary ACTH cells. Namely, EDP decreased while HCG increased the mentioned features in neonatal female rats that were killed during the peripubertal period.

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