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GONADOTROPHS IN LONG-TERM OVARIECTOMIZED RAT FEMALES TREATED WITH LUTEINIZING HORMONE RELEASING HORMONE AND ESTRADIOL

MIRJANA LOVREN, MILKA SEKULIĆ, VERICA MILOŠEVIĆ and NATAŠA RADULOVIĆ

Institute for Biological Research "Siniša Stanković, Belgrade, Yugoslavia

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The effects of multiple estradiol dipropionate (EDP) treatment, high doses of luteinizing hormone releasing hormone (LHRH) and the combination of both on immunohistochemical and morphometric parameters of gonadotrophic cells in long-term ovariectomized (ovx) rats were studied using rabbit anti-rat β-follicle stimulating hormone (β-FSH) and β-luteinizing hormone (β-LH) sera and a peroxidase-antiperoxidase (PAP) immunohistochemical procedure. A morphometric method for determining changes of gonadotrophic cell volume, as well as of their number and relative volume density was used. All examined morphometric values in treated females showed a significant decrease in comparison with the ovx controls, the most significant decrease being observed in EDP and EDP+LHRH-treated rats. These results indicate that besides inhibitory action of LHRH and a singificant inhibitory effect of EDP, the combination of both expressed a prominent inhibitory effect on the examined parameters of gonadotrophic cells in long-term ovx females.

Key Words: gonadotrophic cells, ovariectomy, LHRH, EDP, immunohistochemistry, stereology

INTRODUCTION

The secretion of pituitary gonadotrophins is controlled by the hypothalamic luteinizing hormone releasing hormone (LHRH) and is modulated by the feedback effects of gonadal steroids both at the level of the hypothalamus and of the pituitary gland (Fink, 1979). Pau et al. (1986) indicated that ovariectomy enhances the pulsatile release of hypothalamic GnRH and pituitary LH and FSH. Replacement of estradiol-17β (E_2) at the time of ovariectomy prevented the GnRH increase and gonadotrophin secretion. In female rats estrogen can increase the responsiveness of the pituitary gland to LHRH (Fink, 1979), although paradoxically, ovariectomy also increases the pituitary responsiveness to LHRH (O'Conner et al., 1980). Gonadal steroids appear to be less

effective in suppressing plasma LH if there is a delay in their admistration following ovariectomy (Karla, 1985).

The suppression of gonadotrophins following LHRH agonist treatment depends on the regimen of administration (Adams et al., 1986). Continuous infusion of GnRH, particularly at high concentrations, has a suppressive effect on gonadotrophic activity, i. e. constant GnRH stimulation induces internalization and degradation of GnRH receptors on the surface of gonadotrophic cells and, thus, reduces the ability of the cells to respond to continued GnRH-inputs. However, disparity between gonadothropic respansiveness and GnRH-induced desensensitization involves post-receptor loci (Wise et al., 1984). This suppression is even greater in the presence of physiological estradiol concentrations (Schuiling et al.,1987).

The purpose of the present study was to examine morphometric changes of pituitary gonadotrophs after multiple treatment with EDP and LHRH applied alone or in combination to long-term ovariectomized rats.

MATERIALS AND METHODS

Animals. Female Wistar rats maintained in a 12/12 h hight-dark cycle and at 22±2°C, with free access to food and water, were ovariectomized at 12 weeks of age (b. w. about 200 g). The animals were divided into five groups, each comprising seven females. The first group was receiving i.p. 250 μg estradiol dipropionate (EDP) per day for 4 weeks; the second group was treated s.c. with 25 μg LHRH per day for the last three days of 4 weeks; the third group was treated as the first one and was additionally injected with LHRH by the scheme for the second group; females in the fourth group represented long-term ovx controls injected with sterile olive oil and the fifth group consisted of nonovariectomized females which were injected with sterile olive oil for 4 weeks. All females were weighed and killed under ether anesthesia 24 h after the last injection.

Tissue preparation and immunocytochemistry. Pituitary glands were removed immediately after the sacrifice, fixed in Bouin's solution and embedded in paraffin. Serial 5 μm thick sections were used for immunocytochemistry. The pituitary gonadotrophs were identified by the peroxidase-antiperoxidase (PAP) method (Sternberger et al., 1970). The endogenous peroxidase activity was blocked by methanol and hydrogen peroxide for 30 min. Reduction of nonspicific background staining was achieved with normal porcine serum (1:10) for 45 min. The following sequence of antisera was applied: rabbit anti-rat LH-β 1:2000; rabbit anti-rat FSH-β, 1:200; swine anti-rabbit IgG, 1:100. These antisera were kindly donated by Dr A. F. Parlow, NiH, Bethesda, MD, U.S.A. Binding sites were visualized by applying 3,3'-diaminobenzidine (Serva). The sections were counterstained with haematoxylin. For controls the primary antibody was replaced with phosphate buffered saline.

Morphometry. Immunocytochemically stained sections of pituitaries cut through three tissue levels of the pars distalis were used for morphometric examinations of anti-rat-FSH-β, and anti-rat-LH-β reactive cells with visible nuclei.

These immunoreactive cells were stereologically analyzed by simple point counting (Weibel, 1979). Morphometric parameters (the mean volume of the FSH and LH cell cytoplasm and nuclei, relative volume density, as well as the number of immunoreactive gonadotrophic cells per mm^2), were measured exactly as described previously (Lovren et al., 1996). The data obtained for each rat were used to culculate the group average and standard error of the mean (SEM) and differences were statistically evaluated by Student's t-test.

RESULTS

Relative pituitary weight. The data on the pituitary weight in non-ovx, long-term ovariectomized and long-term ovariectomized rats treated with EDP

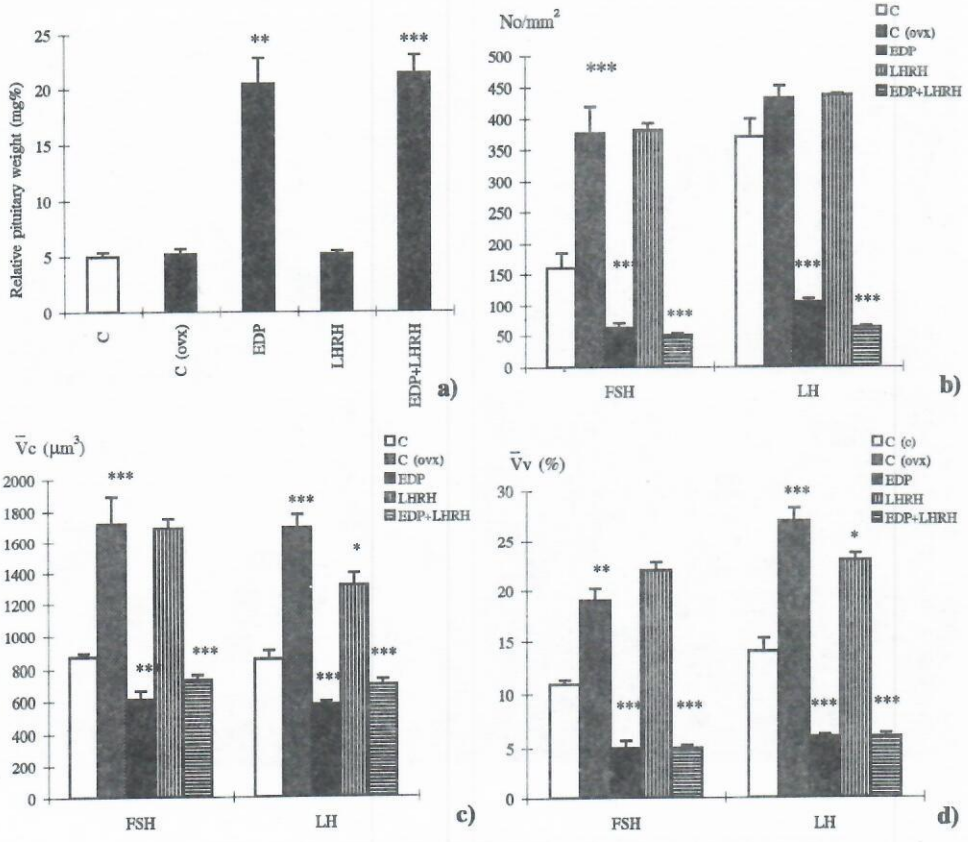

Figure 1. Effects of EDP, LHRH, EDP+LHRH on: a) relative pituitary weight; b) number of FSH- and LH-immunoreactive cells per unit area (mm^2); c) cellular (Vc) volume (μm^3); d) relative volume density (Vvc, %) in long-term ovx females. All values are the means + SEM; *p<0.01, **p<0.005, ***p<0.001.

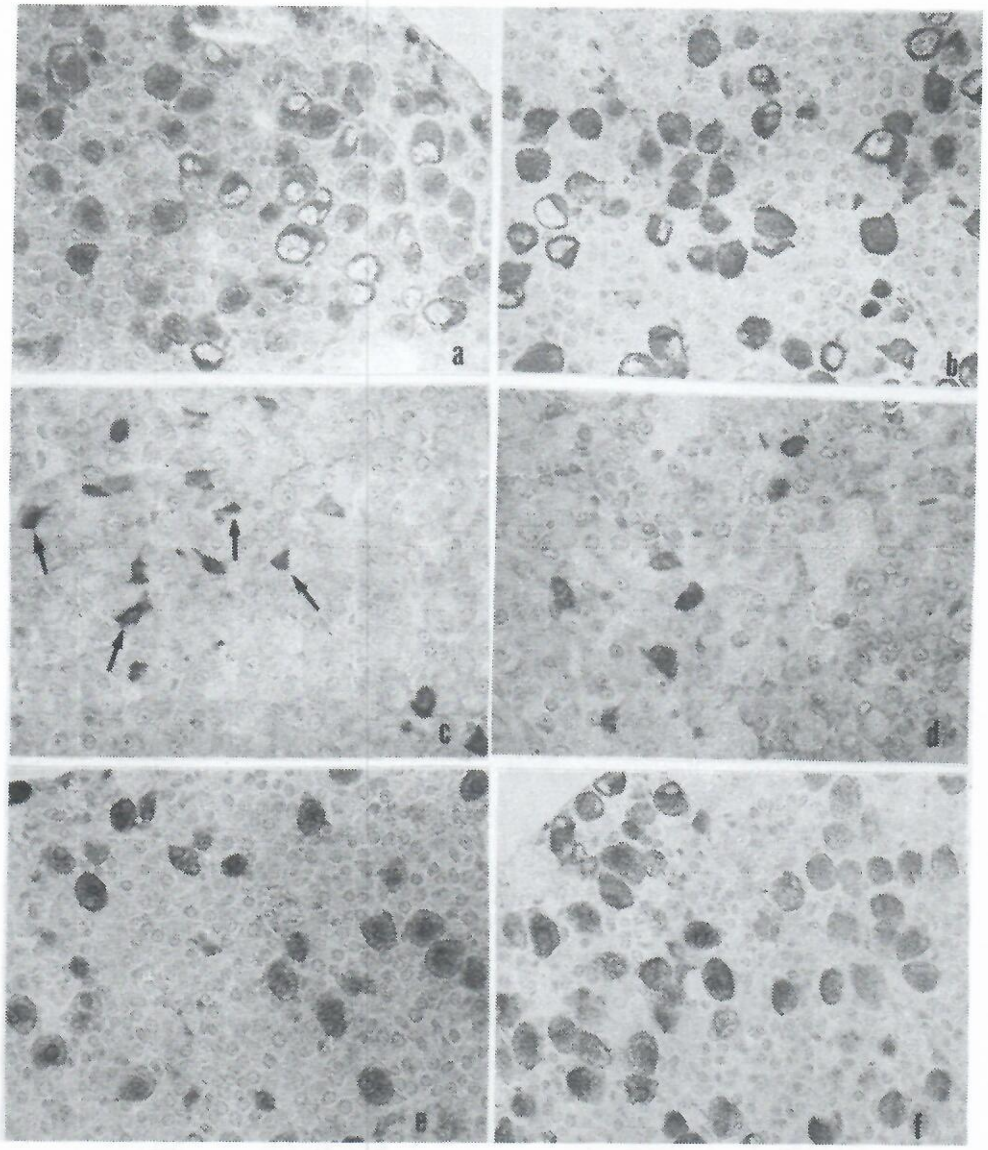

Figure 2. Immunoreacive FSH and LH cells in the pituitary pars distalis of long-term ovx animals: "gonadectomy" FSH- (a) and LH-cells (b) are hypertrophied and vacuolized; long-term ovx EDP-treated rats: a few FSH-, and TSH-like (arrows) cells can be seen (c) and a low number of pycnotic LH cells (d); long-term ovx LHRH-treated females: numerous "restored" FSH- (e) and LH-cells (f). (X650).

and LHRH alone or in combination are given in Fig. 1a. The pituitary weight was not significantly different in long-term ovx females when compared with non-ovx females. In long-term ovx rats treated with EDP alone or in combination with LHRH a significant increase of the pituitary weight was observed in comparison with the ovx controls. No differences in pituitary weight between ovx controls and LHRH-treated ovx females were noticed.

Immunoreactive FSH and LH cells. FSH- and LH-immunoreactive cells of control non-ovx females were round, polyhedral or irregular in shape, some of them being stretched along blood capillaries. Immunostaining of these cells was very strong in the cytoplasm. In this group, FSH cells represented 11% and LH cells 14% of the pituitary volume and had an average volume of 872 μm^3 and 856 μm^3, respectively.

In long-term ovariectomized females, besides hypertrophic FSH- and LH-immunopositive cells, hypertrophic and vacuolated "gonadectomy FSH and LH cells" were present (Fig. 2a, 2b).

These "gonadectomy FSH cells" represented 26% and "gonadectomy LH cells" 22% of the total number of FSH- and LH-reactive cells. In this group volume densities were increased by 73% for FSH cells and by 93% for LH cells in relation to the non-ovx controls. The number of FSH-reactive cells per unit area (mm^2) was increased by 135% and that of LH cells by 17% in relation to non-ovx controls (Fig. 1b). Cellular volumes of immunoreactive FSH and LH cells were increased by 97% and 98%, respectively when compared to the non-ovx controls. Nuclear volumes were decreased in both types of gonadotrophs (21% FSH and 3% LH cells) in relation to the non-ovx controls.

In EDP-treated long-term ovx females "gonadectomy cells" were not noticed. The number of FSH-positive cells per unit area was 6-fold and LH cells 4-fold decreased in relation to the ovx conrols. These few small gonadotrophs showed darker immunocytochemical staining than the controls, suggesting a reduced rate of hormone release. Some of the cells stained for FSH-β, showed morphological characteristics of TSH cells (Fig. 2c, d). Stereological analyses revealed a significantly decreased relative volume density of FSH and LH cells (by 77% and 78%, respectively). Cellular volume of FSH and LH cells was significantly reduced (by 65% and 66%, respectively), as well as nuclear volume (by 21% and 31% and 31%, respectively) in comparison with the controls (Fig. 1c).

In long-term ovx females treated with LHRH most gonadotrophic cells were "restored" so that "gonadectomy FSH cells" represented 9% and "gonadectomy LH cells" 7% in relation to the total number of gonadotrophic cells (Fig. 2 e, f). The number of all FSH-and LH-positive cells per unit area was similar to that in control ovx animals. Relative volume densities of FSH and LH cells were increased by 16% and 15%, respectively. The volume of the FSH and LH cells was decreased by 2% and 22% and the nuclear volume by 34% and 39%, respectively. In ovx females treated with the combination of EDP and LHRH "gonadectomy cells"were not present. FSH-positive cells represented 5% and LH-postitive cells 6% of the pituitary volume. In this group, the number of gonadotrophic cells per unit area was significantly decreased in relation to the ovx controls (FSH cells for 7.5 times,

and LH cells for 6.8 times, respectively). The volume of FSH and LH cells was also significantly decreased (by 57% and 59%, respectively). Relative volume densities of FSH and LH-positive cells were significantly decreased by 77% and 78% respectively (Fig. 1d).

DISCUSSION

In the present study we demonstrated that the most prominent increase in the relative pituitary weight was in EDP- and EDP+LHRH-treated groups. This could be explained by an increased number of chromophobes and LTH cells as reported earlier by Pantić (1980) and Lovren et al. (1996).

It is well established that gonadectomy results in a rise of pituitary gonadotrophin secretion caused by the removalof the negative feedback effects of gonadal steroids. In female rats the response to castration occurs within 39-96 h (Tapper et al., 1972). Such an exaggerated response was observed on all subsequent (6-28) days tested (Whitehead et al., 1982).

In this study we showed that four weeks after ovariectomy, "gonadectomy" FSH and LH cells were "restored" by 74% and 78% respectively. Moreover, total number of gonadotrophs per unit area, their volume density, as well as the cellular volume were significantly increased in relation to non-ovx females.

It is generally accepted that estadiol effects LH and FSH secretion. Administration of estradiol benzoate lowers plasma concenrations of gonadotrophins elevated in long-term ovariectomized rats (Kaira, 1973) completely suppressing the increase of LH secretion (Matt et al.,1986) and partially suppressing FSH secretion (Daklin et al., 1990). Estradiol also modulates gonadotrophin gene expression by direct action on gonadophs or by altering gonadotrophin releasing hormone (GnRH) secreation (Sarker and Fink, 1980). El Etreby and Fath El Bab (1978) indicated that long-term treatment of rats with high doses of estradiol benzonate causes almost complete regression of cells classically identified as gonadothrophs. In our experimental conditions, after EDP treatment of long-term ovx rats, "gonadectomy" cells were not noticed among the few gonadotrophs. The cells stained for FSH-β in the pituitary pars distalis of EDP-treated animals comprise one distent population of cells fitting the morphological description of "TSH cells" i. e. the clasIsical thyrotrophs stored both TSH-β and FSH-β which is in agreement with other studies (El Etreby and Fath Eel Bab, 1978; El Etreby, 1982). In this grup, a reduction in volume density, as well as volume of the cells stained for LH-β and FSH-β was observed. This reduced volume of the cells shown to contain LH-β and FSH-β as a result of EDP treatment suggests suppression of their synthetic activity and probably their secretory function as reported earlier (Lovren and Sekulić, 1993) Also, this finding clearly indicates that EDP within the range of the dose used in this experiment, is sufficient to suppress pituitary gonadotrophic cells.

This is in agreement with the results of Watts and Fink (1984) who reported that estradiol administered to long-term ovx females acts by reducing the amplitude of LH pulses. However, the suppression of pulsatile release is much more effective if estradiol is administered at the time of ovariectomy (Leipheimer et al., 1985). King et al. (1987) found that estradiol was effective in reducing plasma LH levels by 50% in the long-term ovx females. These results were confirmed recently by Arrai et al. (1996) who suggested that this steroid may also be concerned with the regulation of FSH.

The concentration of LHRH in hypophyseal portal blood is increased after gonadectomy (Sherwood and Fink, 1980). Several days after castration, hypothalamic content of LHRH begins to decrease (Kalra and Kalra, 1980). It has been demonstrated that s. c. injection of LHRH decreased the release of LHRH and subsequently LH in ovx rats (Bedran et al., 1985). Also, multiple treatment with LHRH suppressed plasma concentrations of LH and FSH (Bex and Corbin, 1984). The suppression of gonadotrophins following LHRH agonist treatment depends on the regimen of administration: large doses are more effective than low ones, and a constant infusion is even more effective (Nieschlag et al., 1984). Accordingly, our results showed that LHRH treatment of long-term ovx rat females acted by decreasing the volume of both gonadotrophic cell types, although their number was similar to that found in the ovx controls. "Gonadectomy" FSH and LH cells were restored and represented only 9% and 7%, respectively, of the total number of FSHβ- and LHβ-reactive cells.

In this study we also showed that maximal suppression of examined morphometric values of both gonadotrophic cell types was achieved by combined EDP and LHRH treatment. It is well known that in ovx rats, s. c. injections of LHRH decreases the release of LHRH and then of LH (Sarker, 1987). This decrease was dose-dependent, so that large doses were more effective in reducing the plasma concentrations of LH and FSH and depleting the pituitary LH/FSH stores: treatment with EDP lowered the plasma concentration of FSH to about 65% of the control value(Schuiling et al., 1987), i.e. estradiol altered the activity of LHRH neurons, preventing their activity at the level of both the pituitary and the hypothalamus (King et al., 1987). Based on our results and the reports of other authors it can be concluded that multiple treatment of ovx rat females with EDP, leading to increased plasma estradiol concentrations influences the way in which the pituitary responds to high doses of LHRH and may thereby contribute in achieving total suppression of morphofunctional characteristics of gonadotrophic cells.

A c k n o w l e d g m e n t s

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GONADOTROPNE ĆELIJE U DUGOTRAJNO OVARIEKTONISANIH ŽENKI PACOVA TRETIRANIH SA LHRH I ESTRADIOLOM

MIRJANA LOVREN, MILKA SEKULIĆ, VERICA MILOŠEVIĆ, NATAŠA RADULOVIĆ

Institut za biološka istraživanja "Siniša Stanković, Beograd, Jugoslavija

SADRŽAJ

Ispitivan je efekat višekratnih doza estradiol dipropionata (EDP), visokih doza luteinizirajućeg oslobađajućeg hormona (LHRH) kao i kombinacije oba hormona na imunocitohemijske i morfometrijske parametre gonadotropnih ćelija u dugotrajno ovariektomisanih pacova. Za imunacitohemijska istraživanja korišćena je tehnika imunoperoksidaze (PAP), a upotrebljeni su serumi na specifične β subjedinice FSH i L.H. Morfometrijskim metodama su određene

promene volumena, broja i volumenske gustine obeleženih ćelija. Svi ispitivani parametri ukazuju na smanjenje vrednosti morfometrijskih parametara gonadotropnih ćelija tretiranih ženki u odnosu na kontrolnu grupu. Najizrazitije promene su uočene u grupi životinja tretiranih sa EDP i EDP+LHRH. Ovi rezultati ukazuju da pored inhibitornog efekta LHRH i signifikantno inhibitornog efekta EDP, kombinacija ova dva hormona ima izrazito inhibitoran efekat na ispitivane morfofunkcionalne osobine gonadotropnih ćelija u dugotrajno ovaricktomisanih ženki pacova.