0014-4754/95/040335-04$1.50 + 0.20 © Birkhäuser Verlag Basel, 1995

Estradiol-induced changes in TSH-like immunoreactivity of pituitary cells in female rats

M. Sekulić, M. Lovren and T. Popović

Institute for Biological Research, Division of Cytology and Embryology, 11 060 Belgrade (Yugoslavia)
Received 28 March 1994; received after revision 24 June 1994; accepted 12 October 1994

Abstract. Beta-thyrotropin (TSH)-producing cells in the pituitary pars distalis of female rats were studied using rabbit anti-rat beta-thyrotropin (TSH) serum and a peroxidase-antiperoxidase (PAP) immunohistochemical procedure. Animals were neonatally treated with 1 mg estradiol-dipropionate (EDP) and sacrificed at different stages of development up to adulthood. Intact females of the corresponding age served as the controls. Morphometry and stereology were used to evaluate the changes in TSH-cell number and volume densities of the cells and nuclei. All morphometric parameters examined in estradiol-treated animals showed a significant decrease in comparison with immunoreactive TSH cells of age-matched controls. The most prominent EDP-induced changes were evident in peripubertal 38-day-old rats, the number and volumetric densities of both TSH cells and their nuclei being reduced by about 90% compared to intact pituitary. This decrease in the number and volume densities of TSH cells in EDP-treated rats explicitly demonstrated that this hormone, applied neonatally, has an inhibitory effect on TSH-immunoreactive cells up to adulthood, in accordance with our earlier data obtained by light and electron microscopy.
Key words. Female rats; estradiol; pituitary; pars distalis; TSH cells; immunohistochemistry; morphometry.

Gonadotropic cells have been shown to be sensitive to neonatally- and perinatally-administered estradiol in both male and female rats. A subsequent defect in follicle-stimulating hormone (FSH) and luteinizing hormone (LH) regulation within the pituitary during the juvenile and peripubertal periods of life was evident[1–4]. Subcellular organization of TSH cells from the same experimental group (neonatally and perinatally EDP-treated rats) suggested that immature TSH cells postponed differentiation up to maturity[4,5]. The immunohistochemically reactive FSH- and LH-containing cells were smaller in number and size after the neonatal treatment with EDP, appearing to accumulate but not release the hormone content[6]. The latter authors observed no quantitative changes in TSH immunoreactive cells. The existence of cytoplasmic estrogen receptor immunoreactivity in the rat brain has been reported, using three different antibodies directed at three independent sites of the estradiol molecule[7]. Neonatal rat pituitary contains only a small portion of the adult complement of estrogen receptors which are found in a number of cell types[8]. In the rat pars distalis, nuclear concentration of estradiol was observed in all cell types, including acidophils, basophils and chromophobes. More specifically, estradiol localization is not confined to gonadotropes, but occurs also with decreasing intensity in somatotropes, lactotropes and thyrotropes[9].
Estradiol was reported to have a biphasic dose-dependent effect on TSH release, as well[10–13]. This hormone seems to be a significant factor in cell proliferation in the pituitary gland. The highest density in every cell type was seen in females at estrus[14].

The aim of the present investigations, using immunohistochemical and morphometric studies, was to examine whether a high EDP dose administered neonatally would have long term effects on TSH cells of female rats.

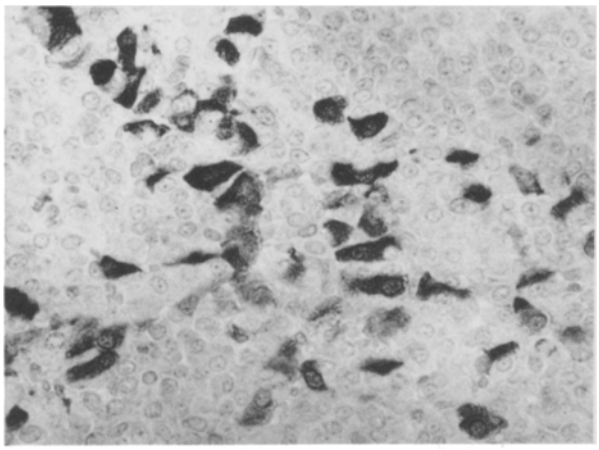
Materials and methods

Newborn Wistar female rats were used. On the 3rd day of life each animal received s.c. 1 mg of estradiol dipropionate dissolved in sterile olive oil (a product of ICN-Galenika Pharmaceutical Works, Belgrade, Yugoslavia). Controls were injected with the vehicle. Animals were sacrificed on days 16, 38 and 80 of life. Each group consisted of five rats.

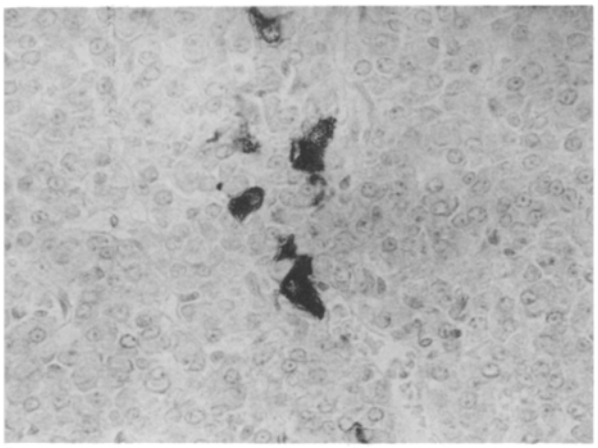
Immunohistochemistry. Animals were anesthesized by ether and decapitated. Immediately after death pituitary glands were removed in Bouin's fluid for 2 days and embedded in paraffin. Serial 4 μm thick sections were deparaffinized in xylene and graded ethanol. For localization of TSH the peroxidase-antiperoxidase complex (PAP) method was used[15]. Slides bearing pituitary sections were placed in a solution of 0.3% hydrogen peroxide in absolute methanol for 15 min to block endogenous peroxidase. After thorough washing with 0.1 M phosphate-buffered saline (PBS) pH 7.4, the sections were incubated with normal swine serum (1:10) for 45 min to reduce nonspecific staining. The following sequence of antisera was applied: rabbit anti-rat TSH beta (kindly provided by Dr A. F. Parlow, Torrance, CA, USA) 1:10,000 for 45 min; swine anti-rabbit IgG (Dakopatts) 1:100 for 45 min. After each step the

preparations were rinsed with PBS for 5 min. Binding sites were visualized by applying 3,3-diaminobenzidine (Serva) in Tris buffer containing 0.03% hydrogen peroxide for 5 min. The sections were counterstained with hematoxylin, dehydrated and mounted. For controls the primary antibody was replaced by PBS.

Morphometry. Immunocytochemically-stained sections of control and EDP-treated rat pituitary glands cut through three tissue levels of the pars distalis were used for morphometric examinations of anti-TSH-beta reactive cells with visible nuclei. TSH-beta-immunoreactive cells were stereologically analyzed by simple point counting. The volume densities of TSH-immunoreactive cells ($V_{vc}/\mu m^3$) and nuclei ($V_{vn}/\mu m^3$) and numerical density of nuclear profiles of TSH cells were determined using 50 test-areas, at a magnification of 1000×, with multipurpose test-system M_{42}. Numerical density of



A



B

Figure 1. *A*) TSH-immunoreactive cells in pituitary pars distalis of control 38-day-old female rat (×716);
B) A few small TSH-immunoreactive cells in the pituitary of 38-day-old rat neonatally treated with EDP (×716).

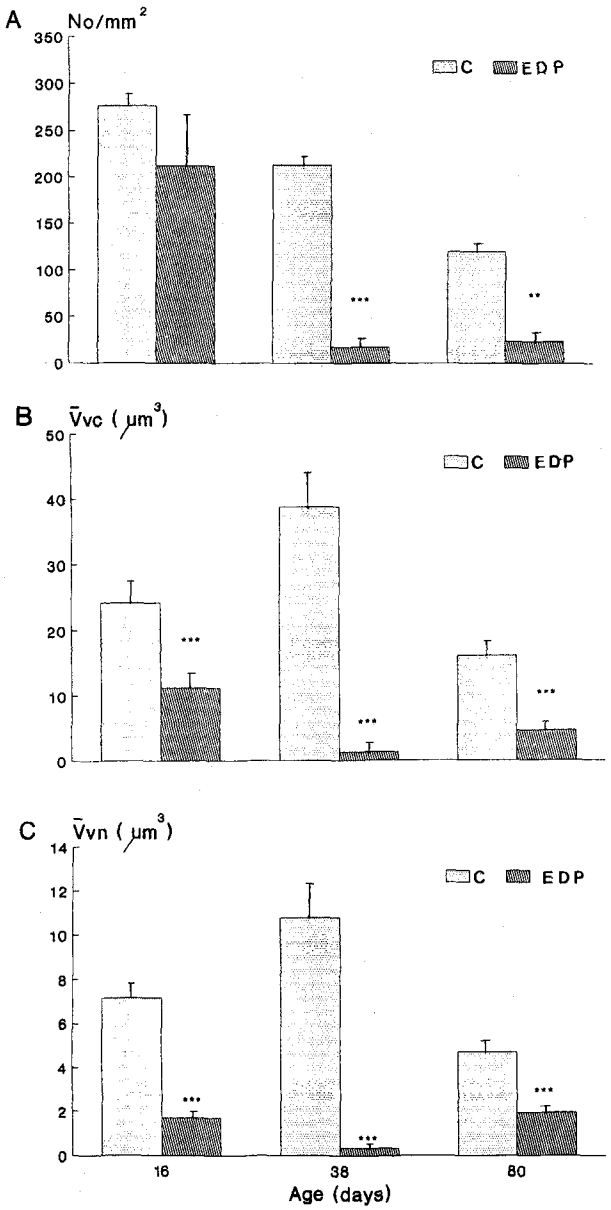


Figure 2. Graph *A*) the number (No) of immunoreactive TSH cells per unit area (mm^2);
Graph *B*) The cellular ($\bar{V}_{vc}$) volume (μm^3) of TSH-immunoreactive cells;
Graph *C*) The nuclear ($\bar{V}_{vn}$) volume (μm^3) of TSH-immunoreactive cells in control (C) and estradiol-treated females (EDP) from 16th to 80th day of life. All values are presented as mean ± SEM. $^{**}p < 0.01$; $^{***}p < 0.001$.

TSH nuclei per mm^3 (and thus of the cells) was estimated according to the formula of Weibel and Gomez[16]. The shape coefficient beta was assumed to be 1.382 for TSH nuclei[16]. At the same time, the number of immunoreactive TSH cells per unit area (mm^2) in each section was analyzed[16]. The data obtained for each rat were averaged per group and standard error of the mean (SEM) was calculated and statistically evaluated using Student's *t*-test.

Results

TSH cells of control females. TSH-immunoreactive cells were preferentially localized in the medioventral portion of the pituitary pars distalis of control vehicle-treated animals. They were round to oval and angular in shape, occuring singly or in clusters and strongly stained. The nuclei were round, vesicular and mostly eccentric (fig. 1A). The number and volume density of TSH cells decreased with age up to the 80th day of life. The decrease in number of TSH cells was accompanied by a reduction in the volume density and the nuclear volume (by 57%, 51% and 34%, respectively), in 80-day-old rats, as compared with 16-day-old animals (figs 2A–2C).

TSH cells of EDP-treated rats. Only a few small weakly stained TSH-immunoreactive cells could be detected in all age groups up to adulthood in rats neonatally treated with EDP (fig. 1B), suggesting a reduced rate of hormone release. A significant decrease in number, volume densities of nuclei and perikarya of TSH cells was noted in treated animals in all examined groups (figs 2A–2C). The most statistically significant ($p < 0.001$) changes were evident in 38-day-old rats neonatally treated with EDP. All these morphometric parameters were about 90% smaller than in control immunoreactive TSH cells (figs 2A–2C).

Discussion

There are only sparse data in the available literature on the influence of estradiol on TSH cell structure and function. It was previously shown that neonatal treatment of the rat with EDP inhibits the ability of gonadotropic cells to synthesise, store and release gonadotropins[1–3]. Also, after neonatal and perinatal treatment of rats with EDP, abnormal TSH cells with unspecific GER and osmiophobic cytoplasm were observed up to adulthood[4,5].

We have found it of interest to examine immunohistochemical and morphometric characteristics of TSH cells up to adulthood after neonatal application of a high EDP dose to female rats, since the pituitary-thyroid axis is not a target for sex steroids. The neonatal period of life is critical for rat development and administration of estrogens during this period has a permanent effect on the hypothalamus, altering development of the nervous system and interneuronal connections[17]. Application of a single EDP dose to neonatal animals provides a convenient model system for further elucidation of the mechanism of its action on the adenohypophyseal cells. It was previously shown that near-physiological estradiol doses given to the rat did not exert an inhibitory effect on FSH cells until the age of 15–20 days[17]. So, it could be supposed that like FSH cells, TSH cells would not be suppressed by low EDP doses. For this reason, high concentrations of this synthetic hormone were applied throughout the present study.

The greatest inhibitory effect of estradiol on TSH-immunoreactive cells was noted in 38-day-old females, neonatally treated with a single EDP injection. The number and volumetric densities of TSH cells and their nuclei in this group were 90% lower than in the control pituitary. In contrast, Vigh et al.[6] observed no effects on TSH cell immunoreactivity after neonatal extradiol application, probably because they used human TSH-beta subunit which did not recognize rat TSH antigens. At the same time, the stimulatory EDP effect was seen as a hyperplasia of chromophobes and LTH cells with an increased rate of synthesis and secretory capacity for prolactin[1].

Complex mechanisms are involved in the developmental regulation of neuroendocrine cells, and gonadal steroids can be applied as modulators in order to control growth, reproduction and behaviour. The characteristics of neuroendocrine cells response to gonadal steroids depends on the dose, as well as on the sensitivity of the cells to a hormone at different stages of differentiation[1]. The general model of estradiol interaction with target cells is also applicable to several types of differentiated cells found in the pituitary. Keefer et al.[9] reported nuclear concentration of estradiol in all cell types of the rat pars distalis, including acidophils, basophils and chromophobes. It was also found that estradiol 17-beta produced a five-fold increase in TSH secretion and a two-fold increase in intracellular TSH concentration in cell cultures[11]. It was suggested that estradiol 17-beta might stimulate TSH secretion by causing proliferation of TSH-producing cells[11] and increasing the density of T_3 and TRH receptors in the rat anterior pituitary gland[18].

On the other hand, D'Angelo[19] reported that increasing doses of estradiol given to female rats produced a progressive decline in TSH concentration both in the pituitary and in the blood plasma, so that TSH level was reduced by 40–90%. This author suggested that suppression of TSH secretion in females given large doses of estradiol probably results from a disturbance in the feedback relationship between thyroid and hypophysis[19]. However, it was demonstrated that estradiol injection to the rats produced a significant decrease in pituitary TSH content, without affecting the release of TSH into the circulation[20]. Zaninovich et al.[21] consider that pharmacological doses of estrogens can acutely depress thyroidal iodine release in healthy subjects and suggested that such an estrogen action could be associated with elevated serum TSH levels. Rutlin et al.[22], however, claimed that estrogens do not affect pituitary TSH secretion.

The data on the presence of estrogen receptors in the hypothalamus[7], pituitary[8] or thyroid gland[23,24] suggest

that this hormone controls thyrotropic function, but the site(s) of its action have not been clearly identified. It should also be mentioned that EDP was shown to inhibit thyroid follicular cells, thus decreasing the level of thyroid hormones[5,25].

The data obtained throughout the present work corroborate and extend our previous findings demonstrating the long term inhibitory action of neonatally applied high EDP dose on TSH cells of the rat pars distalis up to adulthood. It is obvious that the changes in developement of both reproductive organs[2] and thyroid gland[5,25] were achieved by a single EDP injection in a dose far beyond physiological concentrations. This modulatory inhibitory effect of EDP on pituitary cells synthesizing glycoprotein hormones has both fundamental and practical significance, especially for veterinary medicine.

Acknowledgements. We are very thankful to Dr Jelena Joksimović of the Institute for Biological Research, Belgrade, for her continuous interest in this work and help during the preparation of the manuscript. This work was supported by Research Science Funds of Serbia, Grant No. 8000-II.

1 Pantić, V., in: Synthesis and Release of Adenohypophyseal Hormones, p. 335. Eds M. Jutisz and K. W. McKerns, Plenum Press, New York 1980.
2 Pantić, V., in: Reproductive Processes and Contraception, p. 47. Ed. K. W. McKerns, Plenum Press, New York 1981.
3 Lovren, M., Iugoslav. physiol. pharmac. Acta *25* (1989) 137.
4 Lovren, M., Sekulić, M., Hristić, M., and Kalafatić, D., Arch. biol. Sci. (Arh. biol. nauka, Beograd) *41* (1989) 25.
5 Sekulić, M., Acta Vet. Beogr. *36* (1986) 235.
6 Vigh, S., Setalo, G., Török, A., Pantić, V., Flerko, B., and Gledić, D., Bull. T VXI Acad. Serbe Sci. Arts *17* (1978) 1.
7 Blaustein, J. D., Endocrinology *132* (1993) 1218.
8 Sheridan, P. J., and Herbert, D. C., Anat. Rec. *194* (1979) 507.
9 Keefer, D. A., Stumpf, W. E., and Petrusz, P., Cell Tissue Res. *166* (1976) 25.
10 DeLean, A., Ferland, L., Drouen, J., Kelly, P. A., and Labrie, F., Endocrinology *100* (1977) 1496.
11 Miller, W. L., Knight, M. M., and Groski, J., Endocrinology *101* (1977) 1455.
12 Labrie, F., Bargeat, P., Drouin, J., Beaulieu, M., Lagace, L., Ferland, L., and Raymond, V., A. Rev. Physiol. *41* (1979) 555.
13 D'Angelo, S. A., and Fisher, J., Endocrinology *84* (1969) 117.
14 Oishi, Y., Okuda, M., Takahashi, H., Fuji, T., and Morii, S., Anat. Rec. *235* (1993) 111.
15 Sternberger, L. A., Hardy, P. H. Jr., Cuculis, J. J., and Meyer, H. G., J. Histochem. Cytochem. *18* (1970) 315.
16 Weibel, E. R., in: Stereological Methods. Practical Methods for Biological Morphometry, vol. 1, p. 1–415. Academic Press. London 1979.
17 Pantić, V., INSERM (International Symposium on Sexual Endocrinology of the Perinatal Period) *32* (1974) 97.
18 Donda, A., Reymond, F., Rey, F., and Lemarchand-Beraud, T., Acta Endocr. (Copenh.) *122* (1990) 577.
19 D'Angelo, S. A., Endocrinology *82* (1968) 1035.
20 Boado, R., Ulloa, E., and Zaninovich, A. A., Acta Endocr. *102* (1983) 386.
21 Zaninovich, A. A., Boado, R., Ulloa, E., Bromage, N. R., and Matty, A. J., Acta Endocr. *99* (1982) 386.
22 Rutlin, E., Haug, E., and Torjesen, P. A., Acta Endocr. *84* (1977) 23.
23 Marugo, M., Torre, G., Bernasconi, D., Fazzuoli, L., Berta, S., and Giordano, G., J. Endocr. Invest. *12* (1989) 565.
24 Miki, H., Oshimo, K., Inoue, H., Morimoto, T., and Monden, Y., Cancer *66* (1990) 1759.
25 Sekulić, M., Pantić, V., Djurdjević, D., and Milićević, Z., Vet. Glasnik (Beograd) *34* (1980) 443.