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THYROID PARAFOLLICULAR CELLS IN INTACT PIGS, NEONATALLY TREATED WITH GONADAL STEROIDS OR CASTRATED

MILKA SEKULIĆ and MIRJANA LOVREN

Institute for Biological Research, Division of Cytology and Embryology, 29. Novembra 142, 11060 Belgrade, Yugoslavia

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The parafollicular cells (PCs) of male pigs treated with estradiol-dipropionate (EDP) and progesterone (Pr) in combination, or castrated on the first day of life and sacrificed 6 or 8 month later were investigated. The studies included examinations of argyrophil properties, stereological analyses and ultrastructural characteristics of PCs. Treatment with EDP and Pr led to a significant decrease in the number of PCs per unit area, volume density and volume of their nuclei as compared to intact animals (by 25%, 36% and 83%, respectively). Prominently dilated GER and the small number of secretory granules suggested a slower rate of secretion by PCs. Castration led to a decrease in number of PCs per unit area by 18% and an increase of volume density by 5% in 6-month-old pigs in comparison with intact controls. The markedly developed lamellar structure of GER with numerous attached ribosomes and many mitochondria in the cytoplasm of PCs of castrated pigs, suggested stimulation of specific protein synthesis.

Key words: Pigs, parafollicular cells, gonodal steroids, castrates.

INTRODUCTION

Similarly to other mammals, parafollicular i.e. C cells are evident in the pig thyroid gland. They are randomly distributed throughout the gland and contain calcitonin as a hypocalcemic hormone (Blahser, 1978, Pento. 1985). Although the primary secretory product of C cells is calcitonin (CT), they also contain small quantities of calcitonin-gene-related peptide (CGRP). Only a few porcine C cells are immunoreactive to somatostatin (Kameda, 1987). The role of sex steroids in determing PCs structure and function i. e. calcitonin synthesis and secretion, is still poorly understood. Using the silver stain method for detection of argyrophil cells, it was previously reported that EDP applied to rats during the neonatal period, or chronic treatment with this steroid had a stimulatory effect on thyroid PCs which is maintained up to old age (Sekulić and Lovren, 1991, 1992, 1993).

Estradiol replacement therapy plus progesterone in rats or humans protected surgical menopause-associated bone mass loss, i. e. expressed a positive effect on bone formation (Schot and Schuurs, 1990, Slootweg et al., 1992). Both estradiol and progesterone led to a significant stimulation of CT secretion from thyroid C cells of 8-day-old rats in vitro (Greenberg et al.,1986). In was also reported that the administration of Pr alone reduced postmenopausal bone loss, as well (Tremolliers et al., 1993).

On the contrary, Lazaretti-Castro et al. (1991) demonstrated an inhibitory effet of estradiol on CT secretion and content in a human C cell carcinoma cell line. Body (1993) suggested that estrogen replacement therapy in postmenopausal women does not result in a change of bone metabolism.

On the other hand in one-year-old castrated male rats the bone density was significantly reduced 4 months after the operation (Garcia et al., 1989), and the CT level in castrated female rats was found to be decreased (Yamazaki and Kinoshita, 1986). Treatment of ovariectomized rats with 17-β EDP prevented ovariectomy-induced increase of bone resorption and loss (Yamaura et al., 1994). Also, menopause, as a natural event in women, and oophorectomy were risk factors in osteoporosis and reduced serum levels of estradiol, testosterone and dehydroepiandrosterone were found in both events (Otha et al., 1992).

Having in mind the data on the effect of sex steroids or their lack on calcitonin function, it was of interest to examine how neonatal treatment of piglets with a combination of estradiol and progesterone or castration would affect the structure of PCs.

MATERIAL AND METHODS

One-day-old male Swedish Landrace piglets were used. The first group consisted of intact animals. The second group was injected s. c. on the first postnatal day with a combination of 50 mg EDP and 250 mg Pr (products of ICN-Galenika Pharmaceutical Works, Belgrade, Yugoslavia). The third group was castrated on the first day of life. The piglets were left with their mothers until weaning, i. e. up to 36 days of age. Each group sacrificed at the age of 6 or 8 months consisted of 5-10 animals.

The usual industrial feeds for the corresponding age and water were given ad libitum. The piglets were maintained under natural daylight and farm conditions.

The thyroids were quickly excised and pieces of tissue were fixed in Bouin's solution. The paraffin blocks were serially cut into 5 μ m thick sections, staind with haematoxylin and eosin for routine examinations and with the double silver impregnation method to demonstrate argyrophil cells (Pascual, 1986).

For electron microscopy, pieces of thyroid lobe were immersed immediately in 4% glutaraldehyde in Milloning buffer (pH 7.3), postfixed for 1 h in 1% OsO_4 in the same buffer, dehydrated in a graded acetone series and embedded in Araldite. Ultrathin sections were counterstained with uranyl acetate and lead citrate and examined by a Siemens 101 electron microscope.

Morphometry and stereological calculations The sections of each specimen prepared for light microscopy were selected for morphometric evaluations. Morphometric analysis was carried out by point-counting and stereologic calculations were performed (Weibel, 1979). The volume densities of PC nuclei and cytoplasm and numerical density of nuclear profiles of these cells were determined using 50 test areas at a magnification of 1000X, with multipurpose test-system M_{42} (Weibel, 1979). The numerical density of PC nuclei per mm^3 (and thus of the cells) was determined according to the formula of Weibel and Gomez (Weibel, 1979). The shape coefficient β was assumed to be 1.382 for the nuclei. Also, argyrophil PCs were counted in all sections and the number of these cells per unit area (mm^2) in each section was calculated (Weibel, 1979).

The data obtained for each individual were averaged per group and the standard error of mean (SEM) was calculated and evaluated by Student's t-test.

RESULTS

Instact animals The PCs in the pig thyroid appeared to be randomly distributed throughout the gland. Most of these cells occupied a position basal to the thyroid follicular cells or protruded between the latter. They were scattered as single elements, only rarely forming chains. They were of pyramidal shape, elongated and seldom round to oval (Figure 1a). The number and volume density of PCs and their nuclei were less in intact 8-month-old than in 6-month-old animals (Figures, 2a,b,c).

At the subcellular level most of the PCs contained numerous secretory granules in the cytoplasm. These granules were spherical in shape and had an amorphous core-like structure enveloped by a circular limiting membrane. The density of core substance varied somewhat from granule to granule (Figure 3). Also, in some cells many ribosomes and mitochondria and a moderate number of secretory granules were seen in the cytoplasm.

Pigs neonatally treated with female gonadal steroids The small pyramidal or stellate argyrophi PCs were scattered throughout the thyroid gland of the pigs neonatally treated with EDP and Pr in combination (Figure 1b). Morphometric analyses showed that the number of PCs per unit area and volumetric density of these cells and their nuclei in both examined periods were significantly decreased as compared with the corresponding intact animals (Figures 2a,b,c).

In some PCs prominent dilated GER and a small number of secretory granules were seen. The other cells contained numerous lysosomes and changed mitochondria (Figure 4).

Castrated pigs Parafollicular cells of castrated 6- or 8-month-old animals were light and degranulated (Figure 1c). Morphometric studies showed a significant decrease in the number of PCs per unit area and a significant increase of volume density of these cells compared to intact 6-month-old animals. In

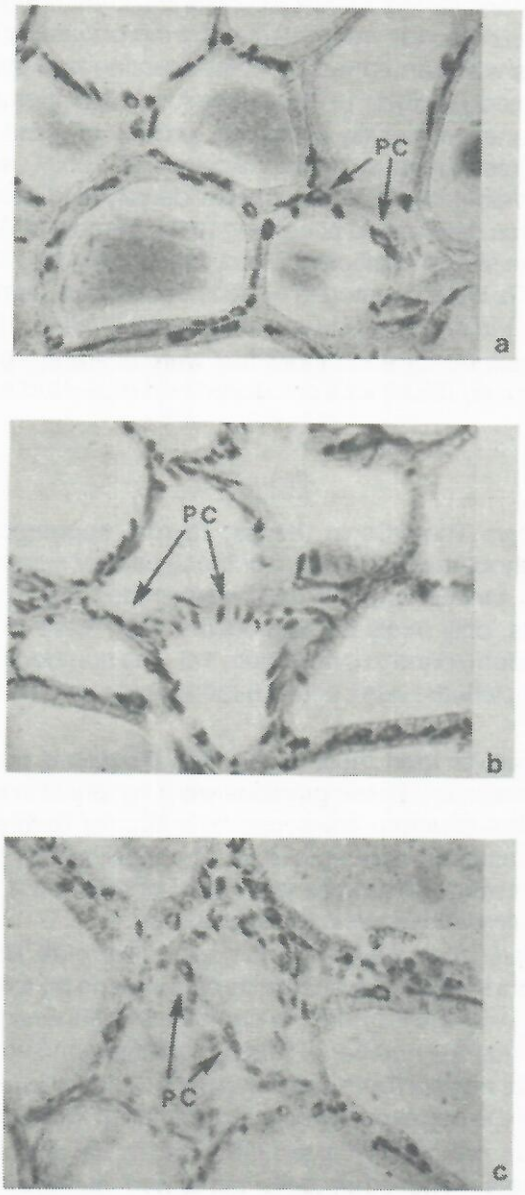

Figure 1. The PCs in the thyroid gland of 6-month-old pigs. a) The basal position of single PCs with long protrusions in control animals;b) The small pyramidal or stellate argyrophil PCs in the thyroid of a pig neonatally treated with EDP+Pr; c) Degranulated PCs of castrated pigs (Pascual double impregnation method; X650).

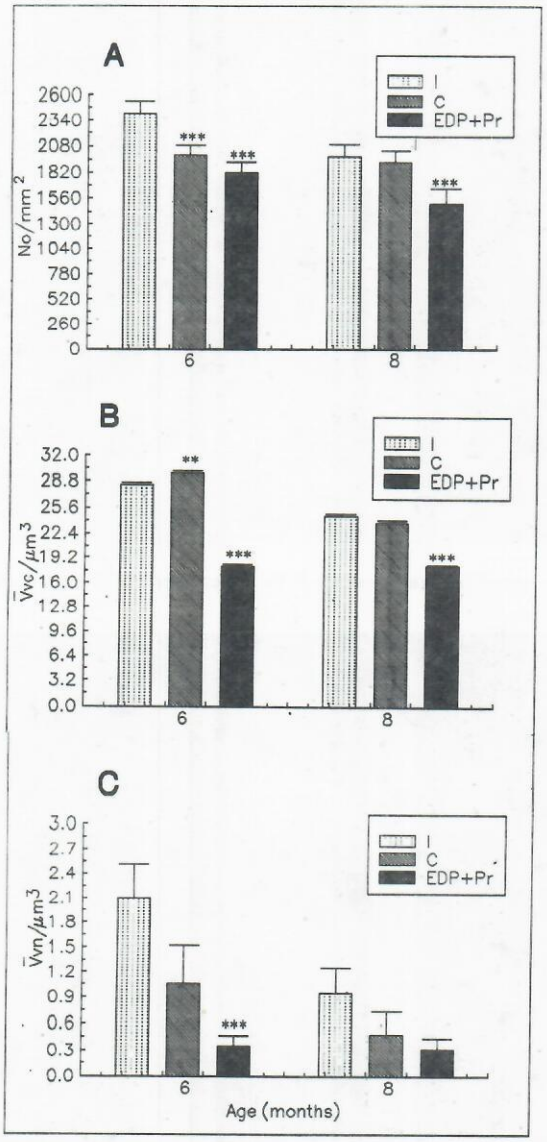

Figure 2. Graph A; The number (No) of PCs per unit area (mm^2); Graph B; The cellular (V_{vc}) volume (μm^3) of PCs; Graph C; The nuclear (V_{vn}) volume (μm^3) of PCs in intact (I), castrated (C) and 6 and 8 months old animals neonatally treated with estradiol and progesterone (EDP+Pr). All values represent the mean ± SEM; *** $p < 0.001$, ** $p < 0.01$.

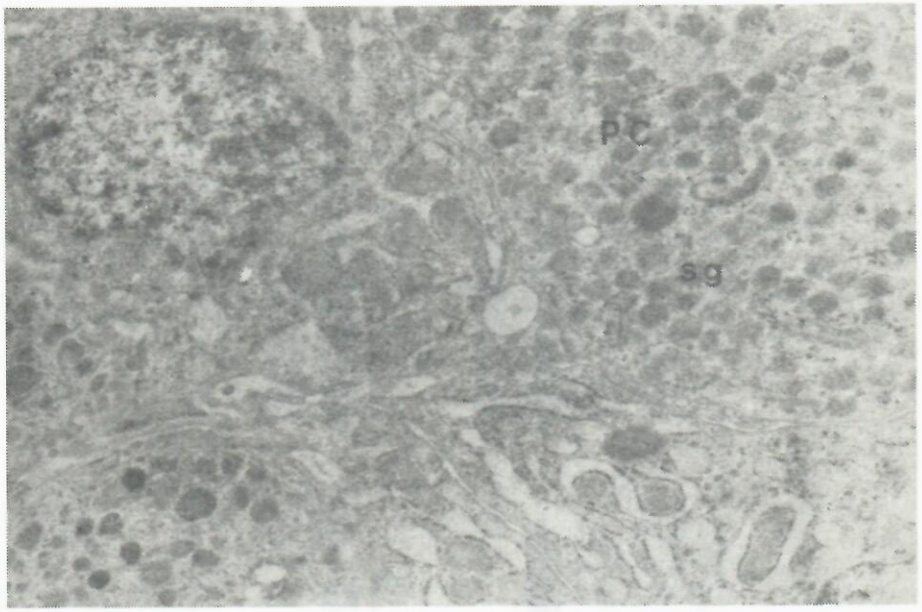

Figure 3. Numerous secretory granules (sg) in the cytoplasm of PC from an intact 8-month-old pig (X20000).

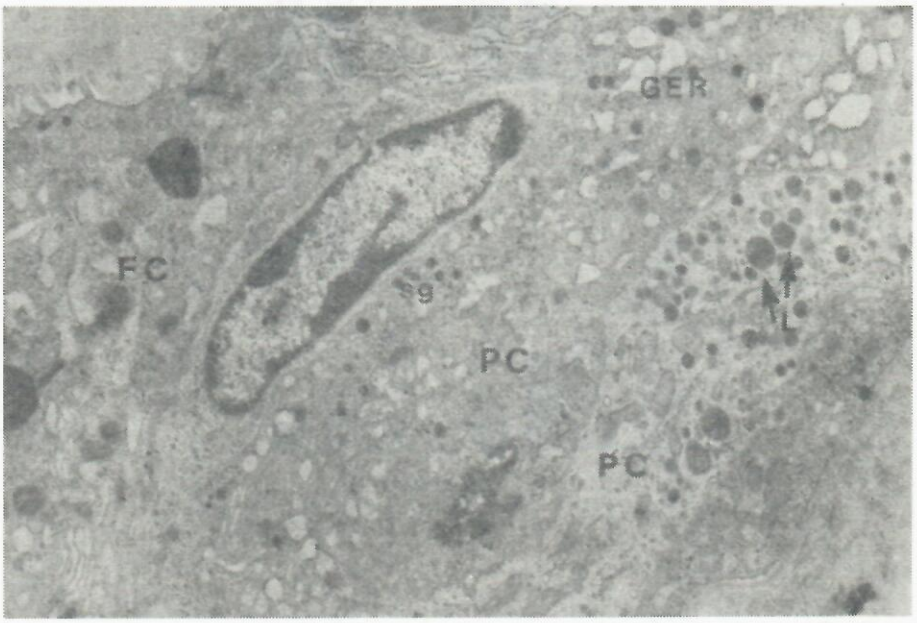

Figure 4. Dilated GER, small number of secretory granules (sg) and presence of lysosoms (L) (arrows) in PCs, located adjacent to follicular cells (FC) of a 6-month-old pig neonatally treated with estradiol and progesterone (X16000).

8-month-old castrated pigs these values were very similar to those of intact pigs of the same age (Figures 2a, 2b). The volume density of the nuclei of PCs from castrated pigs was decreased in comparison with the values in intact animals, but these differences were not statistically significant (Figure 2c).

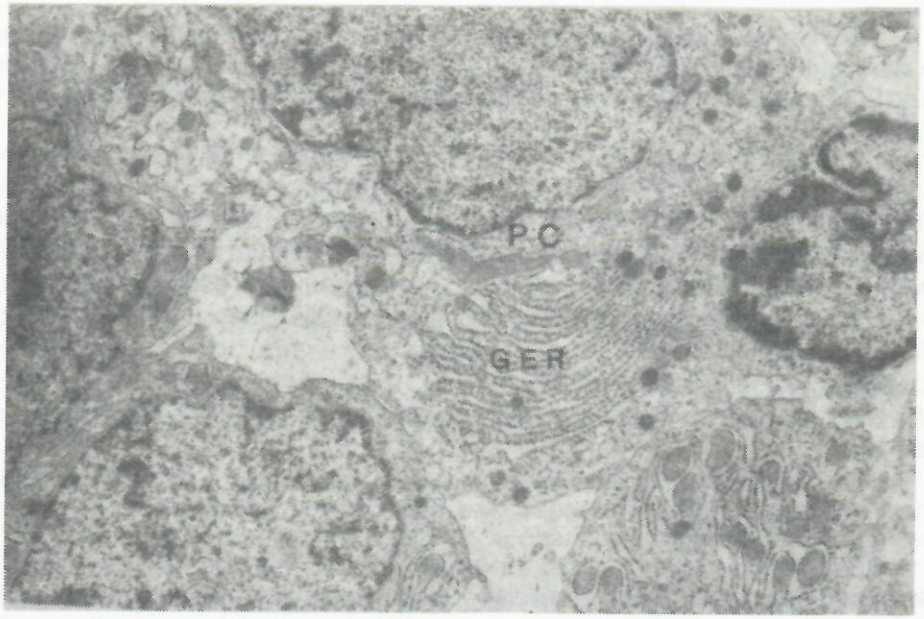

Figure 5. Lamellar arrangment of GER with numerous attached ribosomes in a PC from an 8-month-old castrated pig (X12500).

At the ultrastructural level in both age periods, PCs had a markedly developed lamellar structure of the GER with numerous attached ribosomes and many mitochondria in the cytoplasm. Secretory granules were reduced in comparison with the controls and frequently gathered at the periphery of the controls and frequently gathered at the periphery of the cells (Figure 5). In other PCs the cytoplasm was rich with electron-transparent secretory granules.

DISCUSSION

Porcine PCs are smaller (Blahser, 1978), than rat PCs (Blahser, 1978, Sekulić and Lovren, 1991, 1992, 1993). However, the subcellular organization of these cells is very similar in all mammalian species (Nunez and Gershon, 1978).

In control pigs the majority of PCs, wich were filled with numerous secretory granules with an electron dense core, seemed to be in a "storage" phase of the secretory cycle, as suggested earlier by Capen and Yang (1969).

Our results clearly demonstrate the effect of gonadal steroids on the pig PCs. A decrease in the number of the PCs accompanied by a reduction of the volume density and nuclear volume (by 25%, 36% and 83%, respectively) in

estradiol- and progesterone-treated pigs as compared to intact animals, were observed. The dilated cisternae of GER, the smaller number of secretory granules and numerous lysosomes in PCs of these animals suggested a slower rate of protein synthesis and secretion. Lysosomes may be required to remove secretory granules as they accumulate in the cytoplasm of these cells (Nunez and Gershon, 1978).

Our previous studies on the neonatal influence of EDP on rat PCs revealed a stimulatory effect of this hormone on the function of these cells up to adulthood and EDP-related changes were visible in aged animals (Sekulić and Lovren, 1991, 1992, 1993).

It has been suggested that female gonadal steroids may be involved in the regulation of calcitonin level (Hillyarde et al., 1978), thus influencing the bone cells (Stevenson et al., 1981). Hence, estrogen replacement therapy supplemented with Pr is currently highly recommended for the treatment of postmenopausal women. Progesterone addition also may augment the beneficial effects of estrogens in providing protection against osteoporosis (Schot and Shuurs, 1990, Slootweg et al., 1992), and Pr alone decreases bone resorption in postmenopausal women (Tremolliers et al., 1993). However, little is known about the exact mechanism(s) of action of Pr and its derivates on bone cells (Komm et al., 1988).

On the other hand, stimulatory effects of estrogen on calcitonin secretion have not been recorded in some studies, and thus, the data remained controversial (Body, 1993). Lazaretti-Castro et al. (1991) observed no stimulatory but a transient dose-dependent direct inhibitory effect of this hormone on both CT secretion and content on total cellular protein. These discrepancies presumably reflect differences in the estrogens, experimental schedules applied and CT assays.

The presence of specific steroid receptors in normal and pathological-thyroid tissue (Marugo et al., 1989), i.e. outside the reproductive tract as the target organ suggested that these hormones may influence thyroid tissue. The combination of EDP and Pr neonatally applied to 1-day-old piglets expressed a positive effect on thyroid follicular cells as judged by the signs of increased activity (Sekulić, 1986).

The exact mechanism(s) by which estrogens influence calcitonin secretion remains unclear, since estrogen receptors have not been identified in thyroid PCs in either malignant and normal thyroid C cells, so far (Frolich et al., 1990). On the contrary, Yang et al.(1988) reported the presence of estrogen receptors in a medullary thyroid carcinoma C cell culture. Also, in 1986 Greenberg et al. demonstrated a direct stimulatory effect of 17β-estradiol and progesterone on CT secretion from the thyroid C cells of 8-day-old rats in vitro. These authors observed that tamoxifen as an estrogen receptor blocking agent did not inhibit estrogen-induced CT release. This finding suggested that estradiol does not regulate calcitonin secretion by C cells via a classical receptor system. Thus, it was of interest to see how the lack of sex hormones i. e. neonatal castration would influence the morphology of pig PCs. The volume density of PCs in castrated

animals was significantly increased as compared to the corresponding controls. Subcellular organization of these cells i. e. the long profiles of GER and intense aggregation of free ribosomes, pale vesicles and fanding low density granules suggested stimulation of protein synthesis i.e. this picture corresponds to the protein "synthesizing" phase of the secretory cycle as suggested by Capen and Yang (1969).

The data of Garcia et al. (1989) indicate that castration of male rats causes bone resorption which also occurs after ovariectomy (Wronski et al., 1985, Shen et al., 1992), and 17β-estradiol treatment prevented this ovariectomy-induced increase in bone loss (Yamaura et al., 1994). Serum estradiol level in the pigs castrated on the first postnatal day was shown to be significantly decreased during the first two weeks of age and after that it approached the control levels (Pantić et al., 1984).

The data presented in this work show that porcine PCs respond to exogenously applied sex steroids or to their lack after castration, but the exact mechanism(s) of the affected processes remains to be elucidated.

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REFERENCES

1. *Blacher, S.* 1978. Immunocytochemical demonstration of calcitonn-containing cells in the thyroid glands of different mammals. *Cell Tiss. Res. 186,* 551-558.
2. *Body, J.J.* 1993. Calcitonin: From the determination of circulating levels in various physiological and pathological conditions to the demonstration of lymphocyte receptors, *Horm. Res. 39,* 166-170.
3. *Capen, C.c., Young, D.M.* 1969. Fine structural alterations in thyroid parafollicular cells of cows in response to experimental hypercalcemia induced by vitamin D. *Am. J. Pathol. 57,* 365-382.
4. *Frolich, A., Christensen, L., Andersen, J.* 1990. Estrogen receptors appear undetectable in the C-cells of the human thyroid gland. *Bone 11,* 393-396.
5. *Garcia, S. A., Abramson, E. C., Gajerdo, H. F., Barengolts, E. I., Kellie, S. E., Kukreja, S. C.* 1989. Effect of orchiectomy on axial and appendicular bone mass in aged rats. *J. Bone Min. Res. 4 (Suppl. 1),* S389.
6. *Greenberg, C., Kukreja, S. C., Bowser, E. N., Hargis, G. K., Henderson, W. J., Williams, G. A.* 1986. Effects of estradiol and progesterone on calcitonin secretion. *Endocrinology. 118,* 2594-2598.
7. *Hillyard, C. J., Stevenson, J. C., Macinture, I.* 1978. Relative deficiency of plasma-calcitonin in normal women. *Lancet. ii,* 961-962.
8. *Kameda, Y.* 1987. Localization of immunoreactive calcitonin gene-related peptide in thyroid C cells from various mammalian species. *Anat. Rec. 219,* 204-212.
9. *Komm, B. A., Terpening, C. M., Benz, O. J., Greame, K. A., Gallegos, A., Korc, M., Greene, G. L., O, Malley, B. W., Haussler, R,* 1988. Estrogen binding, receptor mRNA and biologic response in osteoblast-like osteosarcoma cells. *Science. 241,* 82-84.
10. *Lazaretti-Castro, M., Grauer, A., Mekonnen, Y., Raue, F., Ziegler, R.* 1991. Effects of 17β-estradiol on calcitonin secretion and content in a human medullary thyroid carcinoma cell line. *J. Bone Min. Res. 6,* 1191-1195.

11. *Marugo, M., Torre, G., Bernasconi, D., Fazzuoli, L., Berta, S., Giordano, G.* 1989. Thyroid and steroid receptors. *J. Endocrinol. Invest. 12,* 565-570.

12. *Nunez, E. A., Gershon, M. D.,* 1978. Cytophysiology of thyroid parafollicular cells. *Internat. Rev. Cytol. (eds. Bourne, G. H., Danielli, J. F., ass ed. Jeon, K.W.), Academic Press, New York, San Francisco, London, Vol. 52,* 1-80.

13. *Ohta, H., Masuzawa, T., Ikeda, T., Suda, Y., Makita, K., Nozawa, S.* 1992. Which is more osteoporosis-inducing, menopause or oophorectomy? *Bone Min. 19,* 273-285.

14. *Pantić, V., Gledić, D., Milovanović, O.* 1984. Testicular structureand serum concentration of gonadal steroids in male pigs neonatally castrated or treated with estradiol and progesterone. *Bull. Acad. Serbe Sci. et Arts Classe Sci. Mathemat. et Nat. Sci. T. LXXXVI, 25,* 57-72.

15. *Pascual, J. S. F.* 1976. A new method for easy demonstration of argyrophyl cells. *Stain Technol. 51,* 231-135.

16. *Pento, J. T.* 1985. A method for the evaluation of calcitonin secretion using the isolated perfused porcine thyroid. *J. Pharmacol. Meth. 13,* 43-51.

17. *Schot, L. P. C., Schuurs, A. H. W. M.* 1990. Sex steroids and osteoporosis: Effects of deficiencies and substitutive treatments. *J. Steroid. Biochem. Molec. Biol. 37,* 167-182.

18. *Sekulić, M.* 1986. The thyroids of rats and pigs neonatally treated with gonadal steroids. *Acta Vet. 36,* 235-251.

19. *Sekulić, M., Lovren, M.* 1991. Changes in parafollicular cells of rats neonatally treated with estradiol. *J. Endocrinol. invest. 14,* 577-582.

20. *Sekulić, M., Lovren, M.* 1992. Alterations of thyroid parafollicular cell organization in aged rats neonatally treated with estradiol. *Endocrine Res. 18(4),* 291-306.

21. *Sekulić, M., Lovren, M.* 1993. Effects of estradiol and testosterone on morphology of parafollicular cells in aged male rats. *Jugosl. Med. Biohem. 12,* 23-27.

22. *Shen, V., Dempster, D. W., Mellish, R. W. E., Birchman, R., Horbert, W., Lindsay, R.* 1992. Effects of combined and separate intermittent administration of low-dose human parathyroid hormone fragment (1-34) and 17β-estradiol on bone histomorphometry in ovariectomized rats with established osteopenia. *Calcif. Tissue int. 50,* 214-220.

23. *Slootweg, M. C., Ederveen, A. G. H., Schot, L. P. C., Schoonen, W. G. E. J., Kloosterboer, H. J.* 1992. Oestrogen and progesterone synergistically stimulate human and rat osteoblast proliferation. *J. Endocrinol. 133,* R5-R8.

24. *Stevenson, J. C., Hillyard, C. J., Abeyasekera, G., Phang, K. G., MacInture, I.* 1981. Calcitonin and the calcium-regulating hormones in postmenopausal women: Effect of oestrogens. *Lancet. I,* 693-695.

25. *Tremolliers, F., Pouilles, J. M., Ribot, C.* 1993. Effect of long-term administration of progestogen on post-menopausal bone loss: Result of a two-year, controlled randomized study. *Clin. Endocrinol. 38,* 627-631.

26. *Weibel, E.R.* 1979. Stereological Methods. *Practical Methods for Biological Morphometry (Academic Press, London), Vol 1,* 1-415.

27. *Wronski, T. J., Lowry, P. L., Walsh, C. C., Ignaszewski, L. A.* 1985. Skeletal alterations in ovariectomized rats. *Calc. Tiss. Int. 37,* 324-328.

28. *Yamaura, M., Nakamura, T., Kanou, A., Miura, T., Ohara, H., Suzuki, K.* 1994. The effect of 17β-estradiol treatment on the mass and the turnover of bone in ovariectomized rats taking a mild dose of thyroxin. *Bone Min. 24,* 33-42.

29. *Yamazaki, I., Kinoshita, M.* 1986. Calcitonin secreting property of ipriflavone in the presence of estrogen. *Life Sci. 38,* 1535-1541.

30. *Yang, K., Pearson, C. E., Samaan, N. A.* 1988. Estrogen receptor and hormone responsiveness of medullary thyroid carcinoma cell in continuous culture. *Cancer Res. 48,* 2760-2763.

PARAFOLIKULARNE ĆELIJE ŠTITASTIH ŽLEZDA SVINJA NEONATALNO TRETIRANIH POLNIM STEROIDIMA ILI KASTRIRANIH

MILKA SEKULIĆ i MIRJANA LOVREN

SADRŽAJ

Ispitivene su parafolikularne ćelije kontrolnih i eksperimentalnih mužjaka svinja, tretiranih estradiol-dipropionatom (EDP) i progesteronom (Pr) u kombinaciji ili kastriranih prvog dana po rođenju, a žrtvovanih posle 6 i 8 meseci.

Parafolikularne ćelije su morfološki identifikovane primenjujući specifično dvostruko bojenje srebro nitratom. Rezultati ispitivanja dobijeni su stereološkom i ultrastrukturnom analizom ovih ćelija.

Posle tretiranja životinja sa EDP i Pr u kombinaciji zapaža se značajno smanjenje broja parafolikularnih ćelija po jedinici površine za 25%, volumenske gustine ćelija za 36% i jedana za 83% u poređenju sa vrednostima dobijenim u intaktnih životinja. Jasno izražena dilatacija GER-a i manji broj sekretornih granula u citoplazmi ovih ćelija ukazuju na smanjenu mogućnost sekrecije ovih ćelija.

Kastracija novorođenih svinja utiče na smanjenje broja parafolikularnih ćelija po jedinici površine za 18%, i povećanje volumenske gustine za 5%, u 6 meseci starih svinja u poređenju sa vrednostima dobijenim u intaknih životinja iste starosti. Lamelarna struktura granulisanog ER-a i brojne mitohondrije u citoplazmi parafolikularnih ćelija ukazuju na povećanu mogućnost sinteze specifičnih proteina u ovim ćelijama