UC 577,1; 61 ISSN 0354-3447

Jugoslov Med Biohem 22: 185–200, 2003

Pregledni članak
Review article

PITUITARY THYROTROPIC CELLS ARE AFFECTED BY STEROID HORMONES

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Summary: The pituitary gland is a heterogenous tissue comprised of several hormone-secreting cells most of which are targeted by sex steroids. Our long-term studies were concentrated on the response of rat pituitary TSH cells to gonadal steroids applied to animals of different age. With this goal, we examined immunoreactive and morphometric, as well as subcellular organization of pituitary TSH cells in rats of both sexes after neonatal and perinatal estradiol-dipropionate (EDP) treatment. The results undoubtedly indicated persistent EDP-related inhibitory changes of tyrotrophs up to the adulthood. At the subcellular level, a delayed differentiation of TSH cells was noticed. Besides, a special attention has been paid to the changes in the structure of immunoreactive TSH cells of middle-aged (14-month-old) rat females, chronically treated with EDP, calcium (Ca) or a combination of EDP and Ca. Based on our results it can be concluded that both EDP and Ca act inhibiting the thyrotrophs under the applied experimental conditions.

Key words: rat pituitary, thyrotrophs, estradiol, calcium, immunohistochemistry, ultrastructure

Introduction

Thyrotrophs are basophilic TSH hormone-producing cells occurring in *pars distalis* (pd) and *pars tuberalis* (pt) of the adenohypophysis. These cells are involved in the regulation of thyroid hormones secretion through the hypothalamic-pituitary-thyroid complex. Within the thyrotrope subsets other neuroendocrine peptides are co-localized with TSH hormone- secretogranins/chromogranins, a highly conserved polypeptide termed 7B2, a neuropeptide substance P, neuromedin B, galanin, and calretinin. Based on previously reported data, they may function as local modulators of TSH secretion in an autocrine or a paracrine fashion. However, the precise function of these neuroendocrine peptides in thyrotrophs is yet to be explained.

Hypothalamic neurons secrete multiple regulators of TSH secretion. Among these regulators TRH as a stimulator and somatostatin and dopamine as inhibitors are considered to be of utmost importance. TRH acts to stimulate at first the release and later on the synthesis of TSH, while thyroid hormones (T3 and T4) inhibit these functions in a classic way of negative feedback control. Somatostatin antagonizes the biological effects of TRH in thyrotrophs. This is the main basis of the integrated neuroendocrine control system of the TSH secretion.

The synthesis and secretion of adenohypophyseal hormone TSH is strongly influenced by steroid hormones, as well. The effects of estrogen hormones are mediated through the genome and these steroids strongly influence homeostatic functions and development of the adenohypophysis. Besides the estrogens, the androgens are also involved in determining the cellular composition of the anterior pituitary, but whether the effect of these steroids are direct or mediated *via* the hypothalamus remains to be elucidated.

Calcium plays a pivotal role in stimulus-secretion coupling in endocrine cells. In addition, a rise in cytosolic free calcium concentration represents a key step in signal transduction of a wide range of secretagogues in adenohypophyseal secretory cells.

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Although sex steroids and calcium are widely applied in human medicine, there are sparce data in the available literature concerning their influence on pituitary TSH cell structure and function. Keeping in mind previous experience in this area, the present study was focused on the changes in pituitary TSH cells after a single or chronic treatment of rats of different age with estradiol and calcium, or the combination of both. For this purpose, cytological, histochemical, stereological and biochemical methods have been employed.

Pituitary TSH cells

According to Li (1) basophilic cells producing glycoprotein hormones such as TSH, FSH and LH, synthesize the hormones originating from the same ancestor molecule. Yoshimura et al. (2) described six types of basophilic cells in rat pituitary. This division included the intermediary cell forms between the above three types of cells. Applying immunoelectron microscopy Hirano et al. (3) demonstrated that β-subunits of FSH, LH and TSH are colocalized in the same anterior pituitary cells of mush shrews. These authors termed these cells thyrogonadotrophs and suggested them to be a potential stem or progenitor from which thyrotrophs and gonadotrophs have diversed in more advanced mammals. Horvat et al. (4) described in hypothyroid rats bihormonal thyrosomatotrophs containing GH and TSH in dilated rough endoplasmic reticulum and their secretory granules. These results sugest that one cell-one hormone theory in the adenohypophysis is untenable.

In rat fetuses the first immunoreactive TSH cells were noticed on day 17. of gestation (5), while in murine fetuses they were detected on day 16. of gestation, and no sex-related differences were observed (6). Cluster formation of neogenic thyrotrophs begins on day 3, and the size and incidence of the clusters reach a maximum on a day 10. Thereafter, the clusters break down to give rise to singly scattered neogenic thyrotrophs, as reported by Yoshiro et al. (7). These authors (7) suggested that fetal thyrotrophs are not precursors of neogenic TSH cells, but rather may differentiate from pituitary primordial or folliculo-stellate cells. After the birth, during the period of weaning, at 5 7 days of age, the percentage of thyrotrophs is relatively high. The percentage of thyrotrophs established on day 15. of postnatal life represents 4 6% of the pituitary cell population in adult rats (8). The proliferation of thyrotrophs affects the lifespan of the rat through the activity of the thyroid gland (9). In old rats, a decrease of thyroid weight accompanied by a loss of cellular elements and reduction of pituitary TSH cell activity was reported (10). In contrast, no involution of thyrotroph cells in human pituitary gland in aged individuals was observed (11).

Differentiation of TSH cells in the pt starts earlier than that in the pd, from the day 14. of gestation. Numerous authors demonstrated that the secretory activity of TSH pt-specific cells varies with age. It was found to be the highest perinatally, declining in young rats and increasing again in adult animals (12 15, 18). This early differentiation together with a high secretory activity during the fetal and early postnatal period suggests that the pt TSH cells play a key role in developmental processes. It has been shown that both TSH subunits (TSHα and β) of rat and hamster are expressed in pt-specific cells. Morphologically TSH pt cells are smaller than the thyrotrophs of the pd and do not resemble them (16). The function of the pt TSH cells is still uncertain. Hypophyseal *pars tuberalis* TSH cells are not regulated *via* the classic thyrotroph receptors and their intracellular pathways, but through a novel, photoperiod-dependent mechanism (16). It appears likely that the pt cells are a pacemaker for the differentiation and secretory activity of pd thyrotrophic or gonadotrophic cells (17, 18). In contrast to secretory cells of the pd, the specific pt cells do not respond to functional changes of peripheral endocrine glands by morphological alterations. Also, it is worth mentioning that melatonin receptors were detected on the pt TSH cells of fetal and newborn rats (19, 20). Thus, these cells may relate between the *epiphysis cerebri* and the thyroid or the gonads. The pt-specific cells synthesize a novel glycoprotein hormone, tentatively termed tuberalin, with a distinct β-subunit that varies from one to another species (17,18).

The classic morphological aspect and ultrastructural immunocytochemical observations defined the pituitary pd TSH cells as polygonally shaped with a few small secretory granules (100 150 nm in diameter) along the cell periphery. These are the smallest secretory granules whithin various cell types in the anterior pituitary. A normal thyrothroph contains spherical granules and little cytoplasm, while cell organelles such as the rough endoplasmic reticulum, Golgi apparatus and mitochondria are poorly developed (21 25). Applying immunogold electron microscopy, Ozawa and Kurosami (26) identified three subtypes of TSH cells during postnatal development of rats: immature (between the birth and 10 days of postnatal life), intermediate (between 10 and 30 days of age) and mature ones (over 30 days of age). Immature TSH cells are oval with a few small secretory granules (50 100 nm in diameter) and poorly developed cell organelles. The intermediate subtype TSH cells are polygonal or stellate containing a moderate number of medium sized

secretory granules (80 120 nm in diameter) and moderately or well developed cell organelles. Mature subtype TSH cells are large, polygonal in shape and contain numerous large secretory granules (120 180 nm in diameter) and well developed cell organelles. Besides the TSH, many regulatory peptides are present in specific secretory granules. The secretogranins/chromogranins (Sgs/Cys) were shown to be colocalized with thyrotropin and luteinizing hormone in secretory granules of the bovine anterior pituitary (27). A highly conserved polypeptide termed $7B_2$ has also been reported within the secretory vesicles of the LH/FSH and TSH pituitary cells of rat (28). The substance-P-immunoreactive cells were found to colocalize with a small subpopulation of TSHβ-immunoreactive cells in human and rat pituitary and these results suggest a relationship between the substance-P and thyroid gland function (29, 30). Other neuroendocrine peptides, e.g. neuropeptide-Y, neuromedin-B and galanin have also been identified within the rat thyrotroph subsets (31 33). Thyrotrophs, gonadotrophs and lactotrophs of the rat pituitary were immunocytochemically stained with rabbit anti-TRH sera (34). Calretinin was identified as a calcium-binding protein in the rat thyrotrophs. It may be involved in the release and/or synthesis of TSH in thyrotrophs through the regulation of intracellular calcium level (35). Thyrotrophs also synthesize transcription factor Pit-1, which is shown to be necessary for thyrotroph development and survival (36). Lin et al. (37) proposed two separate populations of thyrotrophic cells designated Pit-1-independent and Pit-1-dependent thyrotrophs. The former are reported to be a transient population existing only in the fetal pituitary, whereas latter population exists in both the fetal and the adult pituitary. Several authors have reported that the development of thyrotrophic, somatotrophic and lactotrophic cells is dependent on the presence of the Pit-1 transcription factor (35, 38, 39).

Thyroid-stimulating hormone (TSH)

Thyroid-stimulating hormone (TSH) is a member of pituitary glycoprotein hormone family, besides LH and FSH. Each molecule of the hormone consists of two noncovalently bound subunits, α and β. The α-subunit is common to all three above mentioned hormones, while the β-subunit is unique for each of them and confers biological specificity of the heterodimer (40 42). TSH-like molecules are found widely distributed throughout the rodent central nervous system. They possess immunologic, chromatographic and biologic activity similar to that of the pituitary TSH (43).

Molecular mass of TSH is 28 kD. It is aproximatly 200 times larger than T_4, a thyroid product, the secretion of which is controlled by TSH. It should be pointed out that at different physiological states qualitatively different forms of TSH are secreted (44).

TSH is synthesized at ribosomes attached to the rough endoplasmic reticulum (RER). The newly synthesized proteins are transported across the RER lumen to the *cis*-most Golgi stack and from it to the next one *trans* side what is mediated by transporting vesicles. While passing through the Golgi apparatus, secretory proteins get post-translationally processed and glycosylated. Microtubules and granule-associated filaments are also involved in the transport of the secretory granules. The modified secretory proteins are concentrated and packed in secretory granules and transported to the cell periphery. The content of secretory granules, i.e. secretory proteins, are released to the cell exterior by the means of exocytosis (45).

Subcellular fractionation studies disclosed that the combination of α- and β-subunits begins in the RER at early developmental stages and that these combining subunits are endoglycosidase H-sensitive. All glycoprotein hormones have a relatively high carbohydrate content and the α- and β-subunits of TSH contain approximately 21% and 12% carbohydrates respectively (46). In the Golgi apparatus, high mannose oligosaccharides are converted to complex oligosaccharides which modulate biological activity of glycoprotein hormones and contain fucose, N-acetylgalactosamine sulfate and sialic acid residues (44).

Both the synthesis and production of all pituitary hormones are shown to be efficiently regulated at the transcriptional level. TSH α- and β-subunits are encoded by distinct genes on different chromosomes and are synthesized as separate peptides from distinct mRNA (11, 42, 44, 47). The α-subunit gene is expressed in the thyrotrophs and gonadotrophs of the anterior pituitary and trophoblast cells of the placenta. In rats, it consists of 4 exons (48) and is 13.5 kb long. The expression of the TSH β-subunit is restricted to the thyrotrophs (49). Rat TSH β-gene consists of 3 exons and is 4.9 kb long (50, 51). Since the α-gene is expressed in gonadotrophs, as well as in thyrotrophs, it may be anticipated that it contains multiple regulatory elements as various hormonal and physiological effectors (42). Based on the results obtained with cell cultures of newborn rat testicles and ovaries, Chaba et al. (52) suggested cross-effect between gonadotrophins (FSH, LH) and thyrotrophin (TSH). TSH β-gene expression seems to be under a tight control of cell-specific transcription factors such as the pituitary factor Pit-1 (53).

It should be mentioned also that the pituitary-derived TSH of different species was reported to have

substantially different biopotencies. So, human TSH was found to be 29- and 10-fold less potent than bovine and rat TSH, respectively (44).

Regulation of thyrotrophin synthesis and secretion

Hypothalamic regulation of TSH

It is generally accepted that hypothalamic regulatory peptides called »releasing hormones« regulate their specific target cells in pd of the adenohypophysis. Secretion of the TSH is regulated by hypothalamic hormones, TRH as a stimulator and somatostatin and dopamine as inhibitors, as well as by circulated thyroid hormones.

TRH is a tripeptide (pyroglutamyl-histidyl-prolineamide) separated and identified by research groups of Schally (54) and Guillemin (55). It is synthesized within hypothalamic »peptidergic neurons« of the thyrotrophic area, that extends from the paraventricular and supraoptic nuclei to the anterior border of the median eminence (56). Merduenthaler et al. (57) showed that TRH-immunoreactive neurons exist not only in the hypothalamus but also througout the central and peripheral nervous system, in endocrine cells and in the anterior pituitary itself. In long-term monolayer cultures, TRH may act as a paracrine or autocrine regulator (58). All these findings suggest that TRH is not only a hypophysiotrophic hormone but an important neurotransmitter and/or a modulator, as well. In parvocellular paraventricular nucleus (PVN) neurons, that are directly influenced by the central epinephrinergic system, it may act as a neuromodulator upon other paraventricular neurons.

At the ultrastructural level the TRH-synthesizing neurons express general morphological features of peptidergic hypophysiotrophic hormone-producing cells of the diencephalon, as reported by Liposits et al. (59). Small subcellular particles containing TRH are present in the hypothalamus of 22-day-old fetuses, whereas large particles containing TRH are visible by the seventh day of neonatal life (60). On the postnatal day 4, the portal vein of rats penetrates the external layers of the median eminence to transport the releasing hormones from the hypothalamus to the anterior pituitary (61). On the other hand, Jackson (38) noticed that the pituitary-thyroid axis is relatively independent of the hypothalamus in neonatal rats, and only after day 10. of life the hypothalamus becomes critical for the regulation of TSH secretion. Before the portal system is established, TRH cannot act directly on the pituitary thyrotrophs, even though they may be sensitive to TRH during the fetal period (62). It has been reported that TRH can cross the placenta of the rat (63) and monkey (64). So, the i.v. administration of TRH to the maternal monkey caused a stronger response in the fetal plasma TSH than in that of the mother.

After one day of the PVN destruction in thyroidectomized rats, a significant decrease in median eminence TRH content with a concomitant decrease of blood TSH level occurred (65, 66). TRH receptors in the anterior pituitary are profoundly regulated by thyroid hormones, but not significantly by TRH itself (65). Donda et al. (67) observed that the density of the TRH receptors was higher in the aged than in the young rats, but no changes in the affinity constant were recorded. A primary physiological action of TRH in the thyrotroph is to control the set-point for thyroid hormone negative feedback on TSH secretion. However, this does not appear to be the sole mechanism by which TRH influences TSH secretion since even 2 months after thyroidectomy, when plasma-TSH concentration had plateaued, TSH level was lower by 50% in PVN comparing to that in sham-operated rats (68).

Hyperthyroidism reduces the hypothalamic release of TRH into the hypophyseal portal system, suggesting that a part of the feedback action of thyroid hormones is exerted at the level of the hypothalamus (69). The central noradrenergic system plays an important role in the regulation of TSH secretion under various physiological conditions (70).

Besides, leptin, a peptide hormone produced by the adipose tissue, was found to lead to an increased TRH expression, as well as to a simultaneous activation of the autonomic nervous system. Little is known so far about a potential feedback regulation between the TRH-producing nerves and the sympathetic and parasympathetic centers in the hypothalamus (71).

The action of TRH on the hypophyseal TSH secretion is very rapid and can be detected already within one minute after the TRH injection (72). Brenner-Gati et al. (73) suggested that TSH release could be probably due to more than one mechanism. The acute effects of TRH were interpreted to be caused by the opening of Ca^{2+} cellular channels and of extracellular Ca^{2+} influx into the TSH cells. On the other hand, a more chronic effect may be a consequence of the activation of phospholipase C by TRH, leading to a cascade of phosphatidylinozitol hydrolysis, inositol 1,4,5-triphosphate (IP3) formation and its interaction with the endoplasmic reticulum stores of calcium, thus leading to a rise in the intracellular Ca^{2+} concentration (74). TRH also activates adenylate cyclase in the pituitary thyrotrophs and mammotrophs, but this is not an important mechanism for the induction of either TSH or prolactin release (75, 76).

In addition to stimulating TSH release, TRH stimulates TSH synthesis in rat pituitary, as well. Ultrastructural and morphometric studies on the rat pituitary revealed that TRH accelerates synchronously both the secretion and the synthesis of TSH (77). This effect is performed primarily by the stimulation of the transcription of both TSH α- and β-mRNA (42), as well as during posttranslational carbohydrate processing (78, 79).

It is also worth mentioning that several authors (80 83) suggested that somatostatin (SRIH) and dopamine, acting at the pituitary level as neurohormones and neurotransmitters, can contribute to the CNS modulation of the TSH release.

The inhibitory action of somatostatin (SRIH) was confirmed by discrete lesions of SRIH neurons in the rat periventricular nucleus of the preoptic-anterior hypothalamus, leading to a transient elevation in GH and TSH secretion (84). Roussel et al. (85) showed that SRIH inhibits TSH response to physiological concentrations of TRH in primary cultures of rat anterior pituitary cells in a dose-dependent manner. Similarly, increased hypothalamic SRIH levels induced by oral glucose administration, were found to suppress TRH-stimulated TSH response in humans (86).

Centrally administered somatostatins (SRIH-14 and SRIH-28) were reported to inhibit TSH cells in rats of both sexes. During our recent studies, the morphometric analyses revealed a decreased volumetric density of TSH-immunoreactive cells in SRIH-treated animals, but this difference was more expressed in SRIH-14-treated animals than in those receiving SRIH-28 (87, 88).

James et al. (89) demonstrated a synergistic action of thyroid hormone thyroxin (T_4) and somatostatin in the control of TSH secretion, expressing both anti-secretory and anti-proliferative effects on pituitary thyrotroph tumours in mice. Somatostatin also decreased pituitary TSH secretion, caused tumour shrinkage when used for the treatment of human pituitary thyrotropinomas and suppressed proliferation of a human differentiated thyroid carcinoma cell line (90, 91).

Biological actions of SRIH-14 are receptor-mediated and specific receptors were found not only in the CNS, but also in the pituitary (92). In the adenohypophysis, SRIH-binding sites were identified on the three target cell types: somatotrophs, thyrotrophs and lactotrophs (93, 94). Coy and Rossowski (95) suggested the presence of several subtypes of somatostatin receptors and successfully cloned five of them.

Since dopamine receptors have been demonstrated in the rat anterior pituitary, it is likely that this neurotransmitter directly exerts its inhibitory influence on the thyrotrophs (96). Dopamine administration has been associated with a decreased mRNA production and a decreased TSH secretion in rat pituitary cells as reported by Shupnik et al. (42). Overall, dopamine was shown to cause a conspicuous decrease in the TSH mRNA levels that reached even 50% comparing to the corresponding control (97). Stimulation of hypothalamic dopamine by thyroid hormones was reported to inhibit TSH and PRL secretion. It was suggested that it could act either within the hypothalamus by inhibiting TRH peptidergic neurones or directly at the pituitary level by inhibiting the thyrotrophs (98).

Peptide hormones control TSH cells and autoregulatory mechanisms of action

The anatomy of the pd permits hormonal paracrine and electrotonic communication. The hormones released into the extracellular space of the adenohypophysis from any epithelial cell reach the capillaries by diffusion and enter them through the fenestrations in their endothelial tubes (99). Also, hormones released by one epithelial cell are free to interact with neighbouring cells that contain appropriate receptors situated in their cell plasma membranes (100).

Numerous neural peptides have been reported to affect TSH secretion. According to Schwartz and Cherny (101), these neural peptides include neurotensin, angiotensin II and endothelin 3. In addition, neuromedin 3, a bombesin-related peptide found in the thyrotrophs, as well as in the hypothalamus, was demonstrated to act inhibiting the TSH secretion through the action at both the hypothalamic and the pituitary level where it may have an autocrine regulatory function (102). Neurotensin (NT) as a neuroactive tridecapeptide secreted from gonadotrophs and thyrotrophs may act in an autocrine or a paracrine fashion and thereby modulate the secretion of other anterior pituitary hormones. When administered intravenously, NT stimulates the TSH secretion, but intracerebroventricular NT injections were found to act inhibiting the TSH secretion as reported recently by Bello et al. (103). Also, Jackson (38) observed an inhibition of the pituitary-thyroid axis by opioid peptides and the alkaloid morphine.

Rondel et al (69) identified TRH-like peptides in the rat pituitary gland, but their biological significance is still uncertain. The novel TRH-related peptide pGlu-Glu-ProNH_2 was isolated by Aschworth (104) from the reproductive tract and pituitary gland of rats, pigs and rabbits in significant concentrations. However, it is not yet clear whether this TRH-related peptide exerts its

effects via the TRH receptor or a second receptor is involved.

Arginine vasopressin (AVP) as a neuropeptide was found to stimulate the secretion of both TSH and ACTH. Namely, based on the increased percentages of TSH, ACTH and ACTH-TSH cells observed after the stimulation by AVP, Childs et al. (105) suggested a direct effect of this neuropeptide on these cell populations. The results of Frawley et al. (106) demonstrated that oxytocin representing another polypeptide hormone attenuated TRH-induced TSH release by a direct action on pituitary cells. Galanin, a 29 amino acid peptide, located in PVN may play an inhibitory role in the regulation of TSH secretion, presumably by affecting TRH secretion (107). It should be mentioned also that Toni et al. (108) observed that neuropeptide Y (NPY), a 36 amino acid peptide, occurs in neurons innervating TRH-synthesizing neurons in the rat hypothalamic PVN.

The 1.25-dihydroxy-vitamin D_3 was shown by Tornquist and Lamberg-Allard (109) to express a stimulative effect on the TRH-induced TSH release in rats, but the exact mechanism of its action remained obscure. These authors suggested that 1,25-$(OH)_2$-D_3 could affect calcium influx or some other calcium-transporting event in the pituitary and affect the TSH release by this route.

Arisawa et al. (110) hypothesized that the substance-P, another polypeptide, could regulate the TSH release in estrogen-primed rats.

Regulation of TSH by thyroid hormones

TSH secretion increases only when plasma thyroxin (T_4) decreases and that occurs when the hormone store in the thyroid is sufficiently depleted (111). Several authors demonstrated that the concentration of thyroid hormones is the most important physiological inhibitor of TSH synthesis and release (42, 112). The inhibitory effects are not merely the results of a direct antagonism of the TRH effects. They can be observed when the hypothalamic source of TRH is destroyed or the pituitary separated from it *in vitro* or *in vivo*. Pekary et al. (113) reported a 66% decrease in basal TRH release by hypothalami of aged rats in vitro, as well as reduced serum T_4 levels, while the serum T_3 and TSH were maintained within the normal range of concentrations. These data suggests that a gradual loss of the essential TRH stimulation of TSH release with ageing may be compensated by a decline in T_4 inhibition of TSH release at the pituitary level. Low circulating levels of thyroid hormones T_4 and T_3 in aged rats may also contribute to an increased density of TRH receptors, since T_3 is known to exert an inhibitory control on pituitary TRH receptors (114).

The ability of thyroid hormones to inhibit TSH secretion is mediated through its effects on the thyrotrophs, TRH neurons and also via the regulation of somatostatin secretion. The results of Peterfreund et al. (115) revealed that thyroid hormones act stimulating hypothalamic somatostatin secretion, while the hypothalamic content of somatostatin is reduced in hypothyroidal rats.

3,5,3'-Triiodothyronine (T_3) derived either by intrapituitary conversion by the type II 5'-deiodinase or directly from the circulating T_4, binds to a specific nuclear receptors as demonstrated by Murakami et al. (116). There are two subtypes of nuclear thyroid hormone receptors (TR): TRa and TRb (117). The TRb2 isoform occurs in both the anterior pituitary and the hypothalamus (118), thus providing a mechanism through which T_3 regulates the TSH and TRH secretion. Attachment of T_3 to TRβ2 activates the T_3-receptor complex that binds to the specific nucleotide sequences referred to as thyroid response elements in the promoter region of the genes for TSH α- and β-subunit (42, 119). This thyroid hormone profoundly decreases transcription of the TSHα and TSHβ genes, but the degree of suppression is greater for the latter than for the former gene.

Calcium-dependent TSH secretion

Besides the above listed hormones, secretory processes in the anterior pituitary are controlled by regulatory molecules such as inorganic ions, calcium, vitamins, metabolites and growth factors (120). Numerous cell functions, e.g. secretion, contraction, excitation, proliferation and differentiation depend on intra- and extracellular Ca^{2+} concentration. In general, cytoplasmic calcium appears to be the principal regulator of secretion in neuroendocrine cells, with cAMP-dependent or protein kinase C-mediated pathways serving to modulate Ca-dependent steps (121).

According to Fisher and Polk (122) and Zorec (123), the release of TSH from the thyrotrophs is a Ca-dependent process. Exocytosis is regulated by extrinsic, as well as intrinsic inputs to the pituitary, by hypothalamic hormones reaching the pituitary via the portal system and by local paracrine or autocrine factors (124).

TRH modulates the action potentials of isolated pituitary cells by increasing the free cytosol Ca^{2+} level. This mechanism is mediated either through the stimulation of calcium influx, or by its mobilization from intracellular stores, or both (125). As the main function

of the modulation of action potential in pituitary cells is linked to signalling via voltage-gated Ca^{2+} channels, their predominant role was presumed by Mollard and Schlegel (126). According to Peters et al. (58) calcium ionophore A 23187 may be involved in Ca influx.

Gillet at al. (127) and Bergenfelz et al. (128) reported decreased TSH levels in individuals after different types of Ca^{2+} treatment. Our recent results clearly showed that chronic treatment of middle-aged rat females whith calcium evoked degranulation in glycoprotein-produced pituitary cells (gonadotrophs and thyrotrophs) and decreased the volume and the number of these cells, what could be interpreted as a sign of their reduced synthetic capacity (129, 130). Besides, our new data indicates a significant decrease of serum TSH after this treatment of middle-aged rat females (to be published).

Steroid hormones are involved in the control of TSH

Sex steroids regulate the TSH activity

Several adenohypophyseal hormone-producing cell types including lactotrophs (131, 132), gonadotrophs (133), somatotrophs and thyrotrophs (134) are known targets for estrogens. Estrogen-induced changes are presumably mediated via specific intracellular estrogen receptors, ERα and ERβ (135). According to Mitchner et al. (136) only 10% of pituitary cells coexpress both ERα and ERβ. These authors suggested that ER heterogeneity contributes to the diversity of pituitary cell responsivness to estrogens.

Miller et al. (137) reported that estradiol 17-β produced a five-fold increase in TSH secretion and a two-fold increase in intracellular TSH concentration in cell cultures and might stimulate TSH secretion by causing proliferation of TSH-producing cells. Estrogens not only affect the synthesis and secretion of different pituitary hormones, but also influence the sensitivity of adenohypophyseal target cells to the hypothalamic neuropeptide TRH (138). In the presence of elevated estrogen concentrations, an increase of TRH receptor level was observed both *in vitro* and *in vivo* and this may account for the heightened sensitivity of pituitary cells to TRH (139, 140). Donda et al. (141) also reported that these hormones acted increasing the density of both T_3 and TRH receptors in the rat anterior pituitary gland.

In normal adult rats, proliferative activity of the anterior pituitary is linked to the circadian changes, oestrus cycle and sex (142). In female rats, the number of TSH-positive cells expressing NT-immunoreactivity, as well as the intensity of histochemical reaction, oscillate during the oestrus cycle. So, they are high during dioestrus and decreased through pro-oestrus, as observed by Bello et al. (103). These authors also suggested that sex steroids may control both the NT synthesis and release by direct actions on thyrotrophs.

Estrogen is widely applied in human medicine for the prevention and treatment of different sympthoms and diseases. Rather than a linear response mediated solely through estrogen-responsive DNA elements, *in vivo* estrogens might simultaneously activate distinct signalling cascades that function as networks to coordinate tissue response to these hormones. Quite recently, Segars and Driggers (143) claimed that this complex signalling system provides an exquisite control and plasticity of response to estrogens at tissue level and undoubtedly contributes to a remarkable tissue-specific responses to these sex steroids. Because of the occurrence of both beneficial and undesirable effects during estrogen treatment, it is of great importance to completely understand their effects on all organs, even those that are not specific therapy targets, among them the hypothalamo-pituitary-thyroid axis. Zaninovich et al. (144) consider that pharmacological doses of estrogens can acutely depress thyroidal iodine release in healthy individuals and suggest that such an estrogen action is associated with an elevated serum TSH levels. D'Angelo (145) reported that increased doses of estradiol given to female rats produce a progressive decline in TSH concentration both in the pituitary and in the blood plasma and as a result the TSH level was reduced by 40 90%. The same author suggested that suppression of TSH secretion in females who received large doses of estradiol probably originate from a disturbance in the feedback relationship between the thyroid gland and the hypophysis. However, Boado et al. (146) demonstrated that estradiol injection produces a significant decrease in pituitary TSH content, without affecting its release into the circulation. Chronic treatment of animals with estrogens results in suppression of expression of all tree gonadotrophin subunit genes LHβ, FSHβ and α-glycoprotein subunit (42). Rutlin et al. (147), however, claimed that estrogens do not affect pituitary TSH secretion. The results of Kulig et al. (148) demonstrating that the treatment of hypothyroid α-subunit knockout mice with 17β-estradiol had no influence on thyrotroph hyperplasia contribute to this hypothesis.

Ovariectomy was shown to affect the function of thyrotrophs, as well. The level of TRH-like peptide pyroglutamyl-glutamyl-prolineamide localized in the rat anterior pituitary gland was significantly increased after gonadectomy in rats of both sexes, while the treatment of gonadectomized rats with testosterone or

estradiol 17-β returned the level of this peptide within the range of normal values. It was concluded that the level of TRH-like peptide in the anterior pituitary gland seems to be regulated by peripheral sex steroids (149). In contrast to this, bilateral ovariectomy (Ovx) induced a dramatic reduction in the number of NT-immunoreactive cells. This effect was completely prevented by the treatment of Ovx rats with estradiol and progesterone (103). Our data suggests that chronic treatment of Ovx rats with pharmacological doses of EDP influences both the morphological and stereological features of TSH cells, as well as the serum level of TSH in an inhibitory manner.

In castrated rats TRH occurs in high concentrations in the ventral prostate and it is regulated in a positive dose-dependent manner by testosterone (150). When rat males received estradiol, the enzymatic activity of TRH-degrading ectoenzyme decreased to 65% of control values. This means that the expression of the adenohypophyseal TRH-degrading ectoenzyme was down-regulated by estradiol (138).

The characteristics of the TSH 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. We have shown previously that neonatal treatment of the rats with EDP results in a reduced number and decreased volume densities of TSH immunoreactive cells (151). At the subcellular level and under experimental conditions applied throughout our studies, a delayed differentiation of TSH cells was noticed. The reduction of dense core vesicles in TSH cells positively correlates with the pronounced reduction of TSH-like immunoreactivity, *i.e*. the changes in the intensity of the TSH-like immunoreactivity of EDP-treated rats could be explained by a diminution of the secretory granules density (152, 153). The results of our earlier studies demonstrated that neonatal treatment of the rats with EDP also inhibited the ability of gonadotrophic cells to synthesize, store and release gonadotrophins (154 156).

Perinatal treatment of rat females with EDP led to a decreased activity of TSH cells up to the peripubertal period of life and these changes were expressed to a lesser degree than in gonadotrophic pituitary cells (157). In addition, under the same experimental conditions an increase of »gap junction« communication between the pituitary folliculo-stellate cells was noticed (158).

The results of our previous studies also demonstrated that chronic treatment of middle-aged rat females with EDP inhibits gonadotrophin secretion and affects morphometric and immuno-histochemical characteristics of FSH, LH and TSH cells (159, 160). In addition, we have observed that EDP acted inhibiting the thyroid follicular cells, thus decreasing the level of thyroid hormones (161, 162).

Glucocorticoids regulate TSH activity

Several authors showed that dexamethasone (DEX), a synthetic glucocorticoid hormone, significantly depressed both TRH- and cold-induced TSH secretion in humans and rats (163 165). Brown and Hedge (166) reported that the effect of DEX on TSH secretion depends on various factors such as the dose, steroid-stimulus interval and stimulus-observation interval. Some studies demonstrated that glucocorticoid hormones may act directly on the hypothalamus regulating TRH release or could primarily affect TSH secretion at the pituitary level (165, 167, 168).

Wilbur and Utiger (167) have claimed that glucocorticoids reduce the TSH secretion by acting at the same suprahypophyseal level and Brown and Hedge (169) showed that DEX can exert potentiating or inhibitory effects on this system at both pituitary and suprapituitary sites.

It is possible that the effect of DEX on pituitary sensitivity is mediated via the changes in T_4 levels (169). However, these authors (170) provided no evidence on increased pituitary responsiveness to TRH due to DEX pretreatment. Contrary to this, Faglia et al. (171) showed that in healthy humans DEX might act directly at the pituitary level by reducing the resposnse of TSH to TRH, while not altering significantly the serum T_4 and T_3 uptake.

TRH-induced TSH release was shown to be inhibited in patients who were receiving glucocorticoid hormones either for prolonged periods of time, or in high doses and this TSH response to TRH was clearly dose-dependent (163). Also, it has been demonstrated that glucocorticoids have a suppressing effect on the TSH response to TRH in young and elderly men (172).

Glucocorticoids decrease hypothalamic prepro-TRH mRNA synthesis both directly and indirectly via somatostatin. However, *in vitro* studies have shown upregulation of the prepro-TRH transcript by DEX in several cell lines. This discrepancy may be explained by the *in vivo* complexity of prepro-TRH gene regulation *vs*. the deafferentiated *in vitro* system (173). Enhanced hypothalamic somatostatinergic and dopaminergic inhibitory activities are involved in the mechanism underlying a reduced TSH response to TRH induced by glucorticoid treatment in healthy humans as recently reported by Coiro et al. (174).

Glucocorticoids suppress TSH secretion and the activity of 5'-deiodinase. Thus, during stress, secretion of TRH and TSH is suppressed, while the conversion of T_4 into the biologically active T_3 is decreased in the pituitary. These could be the reasons why the patients suffering from melancholic depression, anorexia, then highly trained athletes, as well as the patients with chronic, inflammatory diseases have a significantly lower thyroid hormone concentration than age-matched healthy controls (173).

Conclusions

The sensitivity and character of response of pituitary TSH cells to gonadal steroids and calcium has been studied in rats of different age and both sexes. Based on the data presented in this review, the following general conclusions may be drawn out:

The administration of a single high dose of EDP during the critical neonatal period of development produces a long-term effect on the anterior pituitary cells. Neonatally applied EDP to male and female rats acts affecting pituitary immunoreactive TSH cells in an inhibitory manner, as judged by the changes at the level of structure and ultrastructure up to the maturity. At the same time, hyperplasia of chromophobes and LTH cells was observed. Increased number of intercellular communications as »gap junction« between FS cells were also recorded.

In rat females treated perinatally with EDP, pituitary TSH cells showed the signs of regression up to peripubertal period of life. An increased number of chromophobes, LTH and »dark« cells was also evident. In addition, intercellular spaces between the pituitary cells were significantly increased.

Chronic treatment of middle-aged rat females with nearly physiological EDP doses acted by inhibiting the structure of pituitary immunoreactive TSH cells. These changes were evident also in animals treated with EDP and calcium in combination, but to a lesser extent than in those receiving EDP alone.

After chronic treatment of middle-aged rats with calcium, the TSH cell degranulation and decreased volume and number of these cells could be the signs of their reduced synthetic capacity.

It short, pituitary glycoprotein-producing TSH cells respond to steroid hormones applied either as a single injection or chronically to rats of different age. It is clear that estrogens play an essential role in the control of function and differentiation, thus modifying the TSH cell cycle.

Acknowledgement. The author is very thankful to Branka Šošić-Jurjević for kind help in preparing the manuscript. This work was supported by the Ministry for Science, Technology and Development of Serbia, Grant #1710.

DELOVANJE STEROIDNIH HORMONA NA TIREOTROPNE ĆELIJE HIPOFIZE

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Kratak sadržaj: Hipofiza je heterogeno tkivo sastavljeno od nekoliko vrsta ćelija koje luče hormone, a na većinu od njih deluju polni hormoni. Naša višegodišnja istraživanja su bila usmerena na proučavanje odgovora hipofiznih TSH ćelija pacova različite starosti na polne steroide. U tom cilju morfometrijski je ispitivana imunoreaktivnost i subćelijska organizacija TSH ćelija hipofize pacova oba pola posle tretiranja estradiol dipropionatom tokom neonatalnog, ili perinatalnog perioda zivota. Dobijeni rezultati nesumnjivo ukazuju na trajne inhibitorne promene tirotropnih ćelija izazvane estradiolom. Te promene se zapažaju sve do zrelosti. Na subćelijskom nivou je uočena odložena diferencijacija TSH ćelija. Osim toga, posebna pažnja je posvećena strukturnim promenama imunoreaktivnih TSH ćelija menopauzalnih ženki pacova (14 meseci), hronično tretiranih estradiolom, ili kalcijumom, ili kombinacijom estradiola i kalcijuma. Na osnovu naših rezultata se može zaključiti da pri primenjenim eskperimentalnim uslovima i estradiol i kalcijum inhibiraju tirotropne ćelije hipofize.

Ključne reči: hipofiza pacova, tirotropne ćelije, estradiol, kalcijum, imunohistohemija, ultrastruktura

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Received: May 23, 2003

Accepted: June 16, 2003