IS THYROID HORMONE A MODULATOR OF ESTROGEN RECEPTOR IN PORCINE FOLLICULAR CELLS ?

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Objective. To examine whether the action of triiodothyronine on aromatase activity in porcine follicular cells is related to the modulation of estradiol receptor.

Methods. Medium and large preovulatory follicles were incubated in Parker medium (M199) supplemented with 5 % of calf serum as a control medium or with addition of triiodothyronine (T_3; 10^{-9}M), tamoxifen (TMX; 0.1 mM) or T_3+TMX. The media were collected after 48 h, and assayed for progesterone (P4) and estradiol (E2) secretion by RIA.

Results. T_3 added to the medium decreased E2 secretion by both medium and large preovulatory follicles (119.7 % and 123.8 %, respectively; $P<0.05$). In contrast, T_3 increased the secretion of P4 by medium (136 %; $P<0.05$), while decreased the P4 secretion by large preovulatory follicles (123 %; $P<0.05$). The effect of TMX added alone was also dependent on follicular development. Estradiol secretion by medium follicles was 2.5 fold higher ($p<0.01$) than in control and 2.9 fold higher ($P<0.01$) than in T_3 treated cells. In preovulatory follicles basal E2 secretion was not affected by TMX, while 1.2 fold higher ($P<0.05$) secretion compared to T_3 treated cells was noted. On the other hand, TMX suppressed basal P4 secretion in medium and preovulatory follicles 1.5 fold ($P<0.01$) and 1.3 fold ($P<0.05$), respectively. The same phenomenon was observed in T_3 treated cells. TMX added to the culture media decreased P4 secretion by medium follicles 1.8 fold ($P<0.01$) and that by preovulatory follicles 1.3 fold ($P<0.05$).

Conclusions. The reversed T_3 action on estradiol secretion by both medium ($P<0.05$) and large preovulatory ($P<0.01$) follicles in TMX treated follicles suggests the up-regulation of ER by triiodothyronine.

Keys words: Triiodothyronine- Follicular cells- Steroid secretion – Estradiol receptors – Estradiol – Progesterone

Recent data showed a suppression by triiodothyronine (T_3) of estradiol secretion by porcine preovulatory follicles (Maruo et al. 1987; Mochizuki and Maruo 1988; Goldman et al. 1993; Gregoraszczuk and Skalka 1996). We have shown previously that thyroid hormone inhibits aromatase activity in porcine theca cells and granulosa cells collected from preovulatory follicles (Gregoraszczuk et al. 1998). It is well known that estradiol action within the ovary is influenced by its rate of production and by the levels of estradiol receptor (ER) present. As shown by Tetsuka et al. (1998), during preovulatory maturation the expression of P450 arom mRNA is up-regulated serving to drive the preovulatory increase in ovarian estrogen production. However, up-regulation of estrogen synthesis corresponds to the decline of ER mRNA expression. This down-regulation of ER mRNA is accompanied by up-regulation of P450 arom mRNA, thereby driving the preovulatory increase in ovarian estradiol secretion (Tetsuka et al. 1998). The question arose whether the observed decrease of aromatase activity and estradiol secretion

by porcine follicles could be due to the influence of triiodothyronine on ER or, considering the fact that ER and triiodothyronine receptor (TR) can bind to an identical half-site (AGGTCA) of their cognate hormone response elements, could act even by cross-talk between ER and TR.

Materials and Methods

Cell cultures and treatments. Ovaries of prepubertal gilts were obtained from a local abattoir and classified according to Channing and Ledwith-Rigby (1975). The ovaries were collected into a bottle filled with sterilized iced saline and transported to the laboratory. Approximately 15 min elapsed from slaughter to ovary collection. In each experiment six ovaries from three animals were selected for cell preparation. Since each ovary yielded 4-6 follicles, the total number of follicles varied between 24 to 36. Medium and large follicles were obtained from ovaries collected respectively at days 10-12 and 16-18 of estrus cycle as described previously (Gregoraszczuk and Skalka 1996).

Experiments. To examine the influence of exogenous T_3 on steroid secretion by medium and large follicles, whole follicles were isolated from the ovary and incubated in Erlenmeyer flask containing 5 ml of medium according to Gregoraszczuk (1990). The flasks were incubated at 37 °C with constant shaking at 70 rev/min, for 2 days. Follicles were incubated in Parker medium (M199) supplemented with 5 % calf serum as a control medium or with the addition of either triiodothyronine (T_3; 10^{-9} M), tamoxifen (TMX; 0.1 μM) or T_3+TMX. In all cases T_3 was added at the initiation of the culture. TMX was dissolved in ethanol and added to cell cultures so that final concentrations of solvents in the medium did not exceed 0.2 %. The chemicals used were purchased from Sigma Chemical Co. (St.Louis, MO). Every treatment was repeated 3 times. The media were collected after 48 h and assayed for progesterone and estradiol concentration by RIA.

Steroid estimation. Progesterone and estradiol were determined by RIA using Spectria kits (Orion, Diagnostica, Finland). For progesterone the limit of assay sensitivity was 94 pg/ml. The coefficients of variation within and between assays were 5.8 % and 2.9 %, respectively. The mean recoveries were 95.1-103.7 %. The cross-reaction with pregnanolone was 2.9 %. All other tested steroids (5ß-dihydroprogesterone, 20ß-hydroxyprogesterone, corticosterone, testosterone, estrone) showed less then 1 % cross-reaction.

For estradiol the limits of assays were 10.2 % and 2.3 %, respectively. The coefficients of variation within and between assays were 10.2 % and 2.9 % respectively. The mean recoveries were 85.6-108.9 %. The cross-reaction with ethinylestradiol was 1.4 %. All other tested steroids (estrone, estriol, progesterone, testosterone, corticosterone) was less then 1 %.

All data points are expressed as means ±SEM, from at least three separate experiments (n=3) each in triplicate. Significance of differences in steroid concentration between control and experimental groups were compared by analysis of variance and by Duncan's new multiple range test.

Results

T_3 added to the medium decreased estradiol secretion by both medium and large follicles (119.7 % and 123.8 %, respectively; $P<0.05$; Fig. 1). In contrast, T_3 increased the secretion of progesterone by medium follicles (136 %; $P<0.05$), while it decreased progesterone secretion by large preovulatory follicles (123 %; $P<0.05$; Fig.2). The effect of TMX added alone was also dependent on follicular development. Thus, estradiol secretion by medium follicles was 2.5 fold higher ($P<0.01$) than in control and 2.9 fold higher ($P<0.01$) than in T_3 treated cells (Fig.1). In large preovulatory follicles basal estradiol secretion was not affected by TMX, while 1.2 fold higher ($P<0.05$) secretion vs. T_3 treated cells was noted (Fig.1). On the other hand, TMX suppressed basal progesterone secretion in both types of follicles (1.5 fold and 1.3 fold, respectively) in medium and large follicles ($P<0.01$ and $P<0.05$, respectively). The same phenomenon was observed in T_3 treated cells. TMX added to the culture media decreased progesterone secretion by medium follicles 1.8 fold ($P<0.01$) and that by large follicles 1.3 fold ($P<0.05$) (Fig.2).

Discussion

The results of the present study confirm our previous results showing that the secretion of estradiol in both medium and large follicles is suppressed

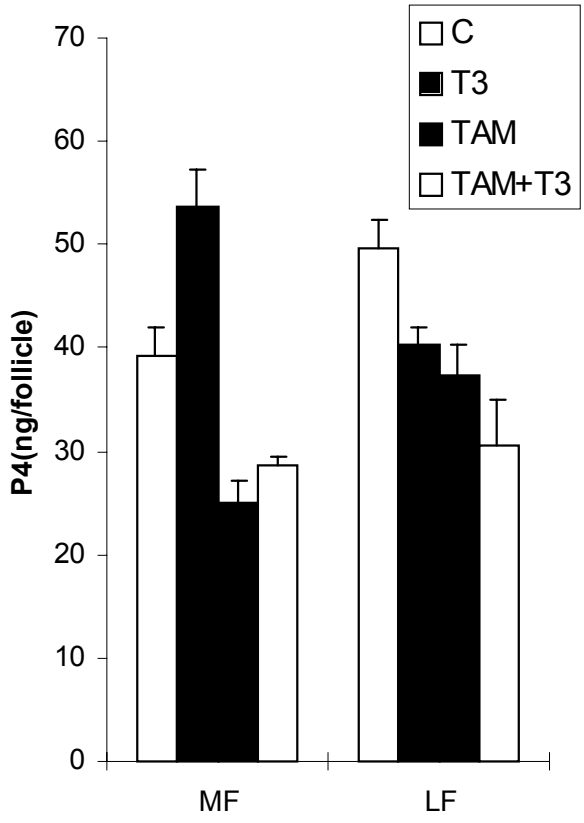

Fig. 1 Estradiol secretion by medium and preovulatory follicles under the influence of triiodothyronine added with or without tamoxifene. T3 – 10^{-9} M triiodothyronine; TAM – tamoxifen.

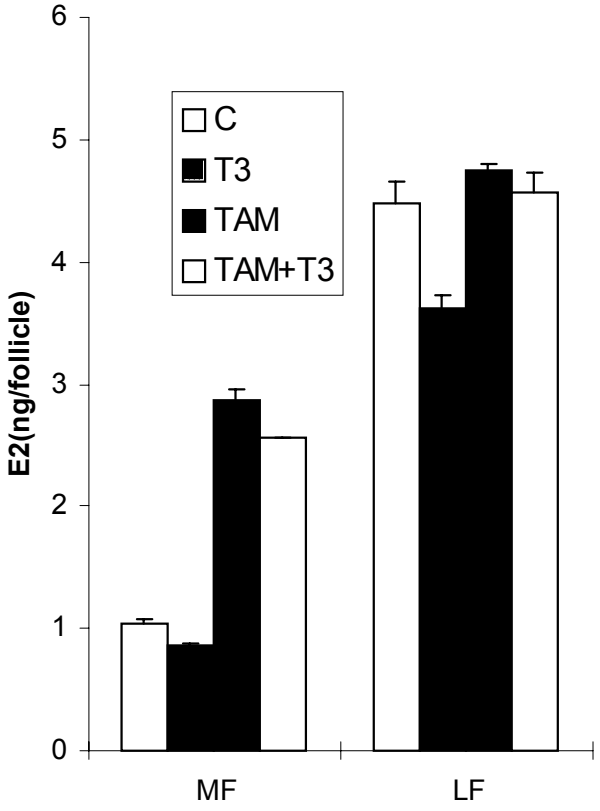

Fig. 2 Progesterone secretion by medium and preovulatory follicles under the influence of triiodothyronine added with or without tamoxifene. T3 – 10^{-9} M triiodothyronine; TAM – tamoxifen.

by triiodothyronine added *in vitro* (GREGORASZCZUK and SKALKA 1996). In addition, the present data showed clear differences in T_3 action on progesterone secretion. Thus, progesterone secretion by medium follicles after T_3 was 1.3 fold higher, while that by large follicles was 1.2 fold lower. This finding suggested a different mechanism of T_3 action on follicular steroidogenesis dependent on the follicular development. The elevated progesterone level in medium follicles under the influence of T_3 could act as aromatase inhibitor which was previously suggested for rat granulosa cells (SCHREIBER et al. 1980) and porcine luteal cells (GREGORASZCZUK 1994).

It seems that in the large preovulatory follicles the mechanism of T_3 action is different. Thus, the decrease in estradiol secretion is apparently coincident with the decrease of progesterone secretion by this type of follicles. SCOTT et al. (1997) investigated the activity of an estrogen response element (EREs) identified in the progesterone receptor (PR) proximal promoter and its interactions with the estrogen receptor (ER) and thyroid hormone receptor (TR) and showed that TR binds to the PR ERE as well as to the consensus ERE sequence in vitro. Further, these two EREs were differentially regulated by T_3 in the presence of TR. High affinity, low capacity T_3-binding sites have been shown in the nuclei of porcine granulosa cells (WAKIM et al. 1987; MARUO et al. 1992). Two types (alfa and beta) thyroid hormone receptors were identified in human granulosa cells (WAKIM et al. 1994). The observed differences in T_3 action depending on follicular development could be due to dependence of T_3 action on the presence of different form of TR. SCOTT et al. (1997) showed di-

vergent pathways existing for activation and inhibition by TR.

In the second part of experiment tamoxifen (TMX), a synthetic E_2 antagonist that inhibits transcription of target gene binding to ER, was used to focus on the effect of T_3 on ER. As high as 2.9 fold increase of estradiol secretion was observed under the influence of TMX added together with T_3 in comparison to T_3 treated cells. This is in agreement with the data by Fukushima and Maeyama (1983) and Groom and Griffiths (1976) showing the increase of follicular phase E_2 levels by tamoxifen.

Daya (1990) suggested that tamoxifen has a direct effect on the ovary to promote folliculogenesis. The reversed T_3 action on estradiol secretion by both medium ($P<0.05$) and large ($P<0.01$) follicles in TMX treated follicles suggests an up-regulation of ER by triidothyronine. The intensity of estradiol secretion under the influence of TMX in medium and large follicles could be interpreted by sufficient doses of TMX used in the present study to block ER in medium but not in large preovulatory follicles. Iwai et al. (1990) reported of weak-to-moderate staining for ER at mid-follicular phase, which becomes more intense in the preovulatory follicles.

Up-regulation of estrogen receptor by triiodothyronine in rat pituitary cell lines was showed by Fujimoto et al. (1997). Other possibility is T_3 action by binding to ER. ER and TR ligand activated nuclear transcription factors that can bind to an identical half-site, AGGTCA, of their cognate hormone response elements (Zhu et al. 1996). These authors suggest that ER and TR may interact to modulate estrogen sensitive gene expression. The presented data showed additionally that TMX decreased progesterone secretion in both control and T_3 stimulated cultures. This inhibitory effect was observed in both medium and large follicles. It could be explained by the fact that antiestrogenic effect of tamoxifen may interfere with estrogen mediated synthesis of progesterone receptors as showed by (Fukushima et al. 1982).

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BOOK REVIEW

NEUROSTEROIDS: A NEW REGULATORY FUNCTION IN THE NERVOUS SYSTERM

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"Steroids are remarkable molecules: basically they look almost alike, being derivatives of cholesterol, but the few slight chemical differences suffice to give them the extraordinary diverse biological specificities that are important in animal physiology and medical therapeutics" – these are words of the Editors of this comprehensive and delighting monograph.

It has been known for a long time that the brain is a target organ for peripheral steroid hormones. However, in 1981 E.E. Baulieu proposed a new term "neurosteroid" which applies to such steroids which accumulate in the central and peripheral nervous system independently of the supply of peripheral endocrine glands and which can be synthesized de novo in the nervous system.

This monographs brings up to date comprehensive review on the present state of art in this new and rapidly developing field. Twenty chapters written by selected experts and carefully edited cover most of major fields of actual interest from molecular biology, biosynthesis and metabolism, mechanisms of receptor transmission and interactions with the receptors of several other neurotransmitters. The distribution of neurosteroidogenic enzymes in the central and peripheral nervous system suggests that neurosteroidogensis appears to be developmentally regulated and that the initial steps of biosynthesis are common to all steroidogenic structures. Special chapters are dealing with cytochrome P450 in CNS, with the key role of steroidogenic factor 1 in adrenal and gonadal development and in endocrine function. Of special interest are several chapters on the distribution and function of individual steroid receptors in CNS, on the neurosteroid binding sites and modulations of the action of benzodiazepine, GABA-ergic, acetylcholine, glutamate and opioid receptors by neurosteroids including either their potentiation or inhibition. Similar modulatory effects of neurosteroids were found also on neuronal voltage-gated calcium channels. From these basal findings further generate the studies on the role of neurosteroids in brain functions starting with their effects on the synaptic plasticity in the brain which is closely related to the presence and distribution of steroid receptors in the brain. Finaly, there are several observations on the effects of steroids on the developing brain, on their memory-enhancing effects and even on several pathways to the future perspectives of promising psychopharmacological profile of neurosteroids and their analogues including the considerations on their membrane and genomic effects.

This monograph will be of great to endocrinologists, biochemists, pharmacologists, neurologists, psychiatrists and all those dealing with any functions of nervous system and its integrative role.

Pavel Langer