TRIIODOTHYRONINE STIMULATES 3β-HYDROXYSTEROID DEHYDROGENASE ACTIVITY IN THE PORCINE CORPUS LUTEUM

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Objective. To study the mechanism of thyroid hormone action on the activity of 3β-hydroxysteroid dehydrogenase in the porcine corpus luteum.

Methods. Pig ovaries were obtained from slaughterhouse animals. Luteal cells were isolated from mid-developing (5-7 days after ovulation) corpora lutea and incubated for 24 h with or without triiodothyronine. Trilostane, an inhibitor of 3β-HSD that blocks the conversion of pregnenolone to progesterone, was added to the medium in doses of 0, 0.1, 1, 10, and 100 μmol. Each treatment was performed in triplicate and each culture system was set up in triplicate. Progesterone concentrations in culture media were determined by radioimmunoassays.

Results. Trilostane in a dose of 100 μM significantly decreased the basal progesterone secretion from luteal cells by 26 % ($P<0.05$). However, such secretion was increased by triiodothyronine (T_3) in a dose of 10^{-9} M. In addition, in T_3-treated cells dose dependent inhibitory effect of trilostane on progesterone secretion was observed. Control cultures grown in control medium revealed a relatively weak 3ß-HSD activity which, however, markedly increased after the addition of T_3 to the culture medium. Trilostane remarkably decreased 3ß-HSD activity in T_3-stimulated cells.

Conclusion. It was found that T_3 acts on luteal cell steroidogenesis via the activation of 3β-hydroxysteroid dehydrogenase in these cells.

Keys words: Triiodothyronine – Corpus luteum – Progesterone – 3ß-hydroxysteroid dehydrogenase – Trilostane

Thyroid hormones are known to exert diverse effects on growth, development and metabolism of nearly all tissues. Possible relationships between thyroid hormones and ovarian steroidogenesis have been repeatedly reported (Goldman et al. 1993; Gregoraszczuk and Skalka 1996; Gregoraszczuk 1996; Gregoraszczuk and Galas 1998). Triiodothyronine (T_3) binding sites have been found in the nuclei of granulosa (Goldman et al. 1993; Wakim et al. 1994a,b) and luteal cells (Bhattacharya et al. 1988). We have recently shown (Gregoraszczuk 1996) an increased progesterone production by porcine luteal cells isolated from early-developing and mid-developing corpora lutea when triiodothyronine was added to the culture medium. Datta et al. (1998) showed that the decrease of thyroid hormone level during the luteal phase of the menstrual cycle (between days 18 and 23 of the cycle) in women is related to a decrease in progesterone secretion.

The enzyme 3β-hydroxysteroid dehydrogenase-isomerase (3β-HSD) converts 5-ene-3β-hydroxysteroids to the 4-ene-3-oxo configuration and therefore plays an essential role in the biosynthesis of hormonally active steroids such as progesterone. Progesterone synthesis by corpus luteum is a complex process involving the uptake, storage and utilisation of cholesterol derived primarily from circulating low-density lipoproteins (Sanders and Stouffer 1995). The initial enzymatic step in the biosynthesis of progesterone involves the conversion of cholesterol to pregnenolone by cytochrome $P450_{SCC}$. Our previous findings have shown that cytochrome $P450_{SCC}$

may be a key target of T_3 action on the mitochondria of luteal cells (Gregoraszczuk and Pieklo 1998). Then, pregnenolone is converted to progesterone by 3β-hydroxysteroid dehydrogenase (Strauss III and Miller 1991).

In porcine granulosa cells *in vitro*, thyroid hormone has been shown to enhance FSH action on aromatase (Maruo et al. 1992; Gregoraszczuk and Skalka 1996; Gregoraszczuk et al. 1998). Earlier work showed the presence of thyroid hormone binding sites in the human corpus luteum (Bhatacharya et al. 1988) and an increase in progesterone production after the addition of thyroid hormone to the porcine luteal cell culture (Gregoraszczuk 1996). These previous studies pointed out to possible multiple sites of action of thyroid hormone on the steroidogenesis.

The current studies were initiated to determine to what extent T_3 regulates 3β- HSD, an enzyme involved in progesterone secretion. For this purpose the classical steroidal competitive inhibitor of 3β-HSD trilostane was tested for its effects on basal and T_3 stimulated progesterone biosynthesis.

Materials and Methods

Reagents. Triiodothyronine (10^{-9} M) was purchased from Sigma Chemical Co (St. Louis, MO, USA). Trilostane (4α,5α,17β)-4,5-epoxy-3,17-hydroxyandrost-2-ene-2-carbonitrile was purchased from Sanofi Pharmaceuticals (Malvern, PA, USA). Medium M199, penicillin, trypsin, and calf serum were from the Laboratory of Vaccines (Lublin, Poland).

Animals and cell isolation. Ovaries were obtained from Large White sows from a local slaughterhouse immediately after slaughter, placed in ice-cold PBS and transported to the laboratory. The phase of the oestrous cycle was determined according to the established morphological criteria (Schilling 1974; Gregoraszczuk 1992). Dissected corpora lutea from each animal were enzymatically dissociated as published previously (Gregoraszczuk 1983). Luteal cells were obtained from pools of freshly excised mature corpora lutea (5-7 days after ovulation) from three animals to produce the luteal cell pool used in any given replicate to minimise the variation possibly existing among corpora lutea in the same ovaries and between ovaries in the same animals and among animals. The cells were suspended in medium M199 supplemented with 5 % of calf serum at a concentration of $3.5x10^5$ cells/ml medium and then grown in multiwell plates (Nunk) in a humidified atmosphere with 5 % CO_2 in the air for 48 h. Incubations were conducted in triplicate in 1.0 ml/well. The cells were cultured with 10^{-9} M T_3. The final inhibitor concentrations used were of 0, 1, 10 and 100 μM. After 48 h incubation, all cultures were terminated and the media were frozen until used for the steroid estimations. Cell viability measured using the trypan blue exclusion test was 85 %. At least three different experiments (n=3), each in triplicate have been performed.

Histochemical evaluation. To evaluate the influence of T_3 on the activity of delta^5,3β-hydroxysteroid dehydrogenase (3β-HSD), the luteal cells were examined histochemically according to Fischer and Kahn (1972); in this histochemical reaction the products of enzyme activity within luteal cells are formazan granules. The reaction was classified as strong (+++), intermediate (++), or weak (+) depending on the intensity of colour. The results were expressed as the percentages of cell classes with different enzyme activity. Five different areas for each treatment were measured and the number of cells was expressed as the mean±SEM.

Progesterone estimation. Progesterone was determined radioimmunologically using Spectria kits (Orion, Diagnostica, Finland), supplied by Polatom (Swierk, Poland). 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 pregnenolone was 2.9 %. All other tested steroids (5β-dihydroprogesterone, 20β-hydroxyprogesterone, corticosterone, testosterone, estrone) showed less than 1 % cross-reaction.

Results

Progesterone secretion. The basal progesterone secretion from luteal cells was significantly decreased ($P<0.05$) by trilostane in a concentration of 100 μM. In contrast, triiodothyronine in a dose of 10^{-9} M increased progesterone secretion from luteal cells (4.7 ng/10^5 cells vs. 3.2 ng/10^5 cells in the control culture). In T_3 stimulated cells a dose dependent

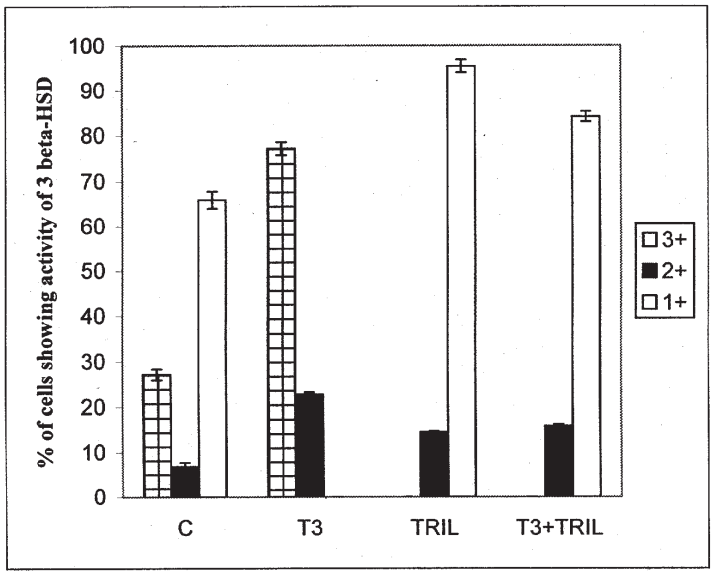

Fig. 1 The influence of trilostane (Tril) in a dose of 1, 10 or 100 μM on basal progesterone (C) and triiodothyronine stimulated (T3) progesterone secretion by luteal cells

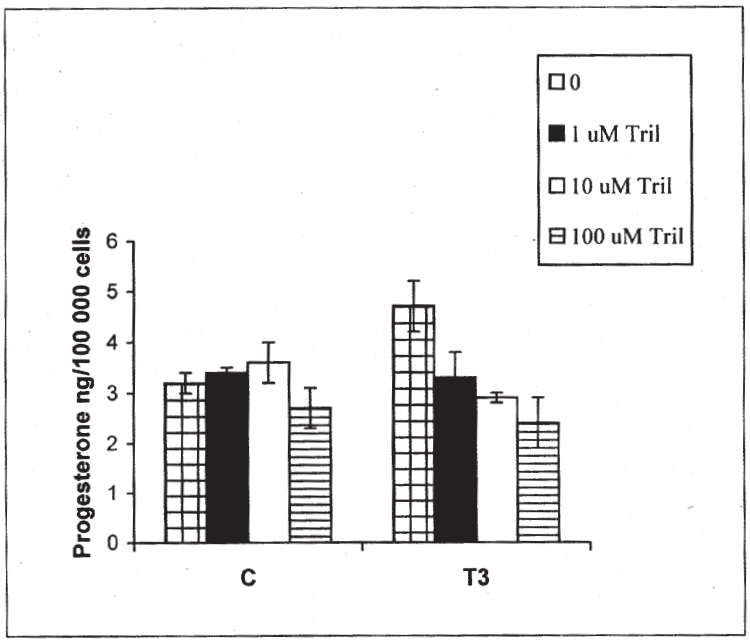

Fig. 2 The influence of T_3 on the activity of delta^5,3ß-hydroxysteroid dehydrogenase (3ß-HSD) of luteal cells. The reaction was classified as strong (3+), intermediate (2+), or weka (1+) depending on the intensity of colour. The results were expressed as the percentage of cell classes with different enzyme activity. Five different areas for each treatment were measured and the number of cells was expressed as the mean±S.E. C – control; T3 – triiodothyronine; TRIL – trilostane

inhibitory effect of trilostane on progesterone secretion was observed (30 %, 39 % and 49 % of basal secretion after trilostane concentrations of 1, 10 and 100 μM; Fig. 1).

Histochemical results. Control cultures grown in M199 medium revealed a relatively weak enzyme activity (Fig. 2a). However, such activity markedly increased after the addition of T_3 to the culture me-

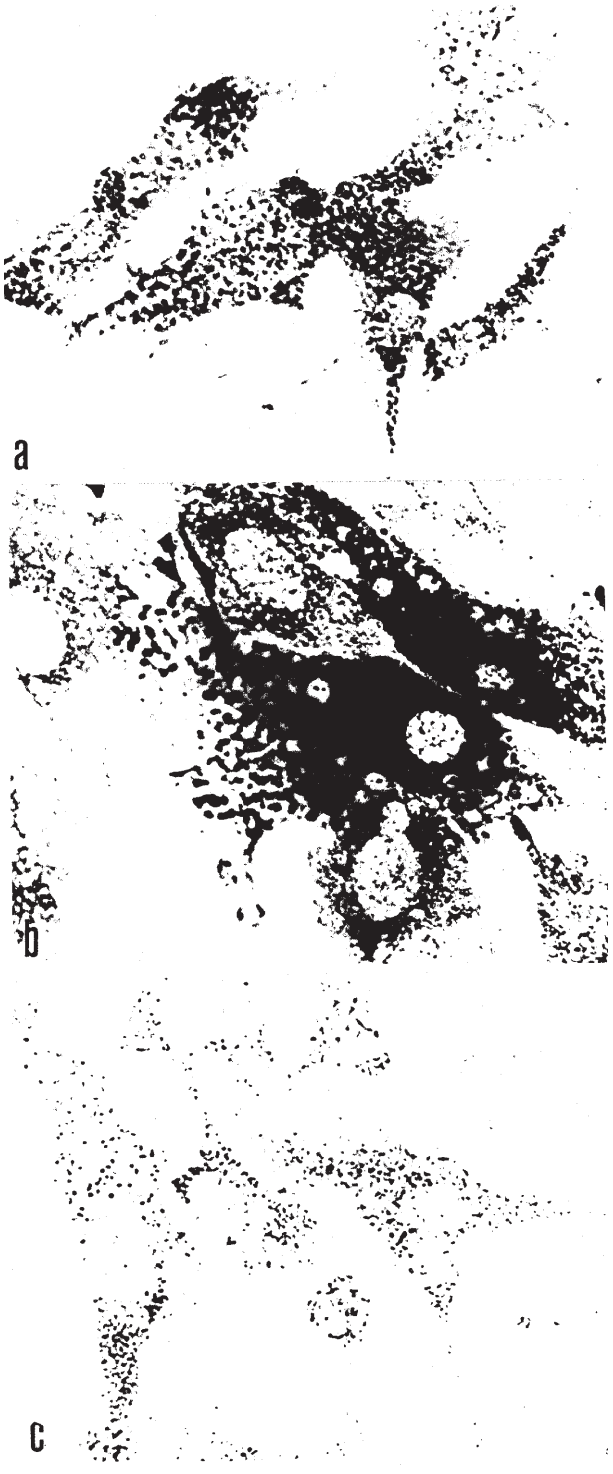

Fig. 3 Luteal cells culture tested for the delta^5,3ß-hydroxysteroid dehydrogenase activity.
a – control cells showing weak histochemical reaction, x 320; b – corresponding culture in medium with addition of triiodothyronine (note strong activity of dehydrogenase), x 320; c – cells cultured in medium with the addition of triiodothyronine+trilostane (decrease of the enzyme activity is visible), x 320

dium (Fig. 2b). Trilostane, the well known inhibitor of 3β-HSD, at a concentration of 100 μM remarkably decreased enzyme activity (Fig. 2c) and, in addition, T_3-stimulated activity of 3β-HSD was inhibited by all doses of trilostane used (Fig.2d).

Discussion

Progesterone synthesis by corpus luteum is a complex process involving the uptake, storage, and utilisation of cholesterol. The initial enzymatic step in the biosynthesis of progesterone involves the conversion of cholesterol to pregnenolone by cytochrome $P450_{SCC}$ (Sanders and Stouffer 1995). Pregnenolone is then converted to progesterone by 3β-hydroxysteroid dehydrogenase (Strauss III and Miller 1991). Previous studies (Gregoraszczuk and Pieklo 1998) have shown that cytochrome $P450_{SCC}$ may be a key target of T_3 action on the mitochondria in luteal cells. On the basis of the current study we hypothesise that 3β-hydroxysteroid dehydrogenase-isomerase plays also an essential role in the triiodothyronine action on luteal cells. Actually, triiodothyronine induced an increase both in progesterone secretion and 3β-HSD activity in cultures of luteal cells. This is in agreement with the data obtained by Bhattacharya et al. (1996) who showed that the addition of T_3 to the fish follicle incubation caused a two-fold increase of ^{3}H-pregnenolone conversion to radiolabelled progesterone as compared to the control. Simonian (1986) showed an interaction between ACTH and thyroid hormone for the stimulation of 3β-HSD activity in human fetal adrenal cortex cultured cells. Trilostane have been shown to be a competitive inhibitor of 3β-HSD by several different sources (Naville et al. 1991; Cooke 1996). The studies presented here demonstrate that in the porcine corpus luteum, this compound also acts as an competitive inhibitor of the 3β-HSD mediated step in progesterone biosynthesis.

The present study demonstrates that trilostane essentially abolished basal progesterone secretion and 3β-HSD activity in cultured luteal cells. Hayashi et al. (1987) using a monolayer culture system of porcine granulosa cells obtained from small porcine follicles showed that thyroid hormone increases FSH-mediated induction of 3β-hydroxysteroid dehydrogenase in immature granulosa cells. Recently pub-

lished observations by DATTA et al. (1999) indicated that the addition of increasing concentrations of T_3 caused a linear increase of 3β-HSD activity.

Steroidogenesis is controlled by a number of factors, the most significant of which are availability of the substrate (MURPHY and SILAVIN 1989), and the effect of the cAMP cascade on cholesterol side chain cleavage (HALL 1985). However, there is good evidence that trophic regulation of steroid synthesis may occur also by the alteration of the activity of appropriate enzymes, including 3β-HSD. Administration of T_3 to the culture medium of luteal cells stimulated 3β-HSD activity over untreated control. Trilostane added together with triiodothyronine reduced progesterone secretion and 3β-HSD activity when compared to cultures treated with triiodothyronine alone.

In summary, the present study demonstrated that T_3 influences steroidogenesis in the corpus luteum by stimulating 3β-HSD activity and this regulation is a component of the luteotrophic regulation of steroidogenesis in the luteal cells. Effective inactivation of 3β-HSD prevents the stimulatory effect of T_3 on progesterone secretion from luteal cells. Further studies are necessary to show if the effect of T_3 on luteal progesterone secretion is direct or, as it shown by BHATTACHARYA et al. (1996) for perch ovarian follicles, by the induction of generation of a novel putative protein in ovarian follicles that stimulates the conversion of pregnenolone to progesterone.

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