Thyroid hormone stimulates progesterone release from human luteal cells by generating a proteinaceous factor

M Datta, P Roy, J Banerjee[1] and S Bhattacharya

Department of Zoology, Visva-Bharati University, Santiniketan-731 235, West Bengal, India and [1]Suri Sadar Hospital, Suri-731 101, West Bengal, India

(Requests for offprints should be addressed to S Bhattacharya)

Abstract

Blood samples collected from 29 women (aged between 19 and 35 years) during the luteal phase of the menstrual cycle (between days 18 and 23 of the cycle) showed that deficiency in thyroid hormone level is related to a decrease in progesterone (P_4) secretion. To observe the effect of thyroid hormone on human ovarian luteal cells, 3,5,3′-tri-iodothyronine (T_3; 125 ng/ml) was added to luteal cells *in vitro*. T_3 significantly stimulated progesterone release ($P<0{\cdot}01$) from luteal cells and this could be blocked by cycloheximide, indicating a protein mediator for the T_3 effect. The T_3 stimulatory effect was inhibited by anti-T_3 antibody suggesting specificity of T_3 action. Addition of T_3 caused a more than threefold increase in cellular protein synthesis which was inhibited by cycloheximide. Preparation of partially purified thyroid hormone-induced factor (TIF) (from peak II of Sephadex G 100 chromatography of T_3-incubated cells), and its addition to luteal cell incubations caused a significant increase in P_4 release ($P<0{\cdot}05$). Incubation with trypsin or treatment with heat destroyed the stimulatory effect of TIF on P_4 release, indicating the proteinaceous nature of TIF. Purified thyroid hormone-induced protein (TIP) from rat granulosa cells and fish ovarian follicles greatly stimulated P_4 release from human luteal cells. These results suggest that T_3 stimulation of P_4 release from human luteal cells is not direct, but is mediated through a putative protein factor, which appears to be a protein conserved through evolution as far as its biological activity is concerned.

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Introduction

The involvement of thyroid hormone in mammalian ovarian (Southren *et al.* 1974, Akande 1975) and testicular (Clyde & Walsh 1976, Kidd *et al.* 1979, Chandrashekar *et al.* 1986) function has been suggested for a long time. Irregularity in menstrual bleeding in hypothyroid women has been shown to be due to the failure of progesterone secretion (Ingbar & Woebar 1981). The existence of thyroid hormone receptors in mammalian gonadal cells (Wakim *et al.* 1987, Bhattacharya *et al.* 1988, Biswas *et al.* 1993, Jana & Bhattacharya 1994) and restoration of impaired steroid hormone metabolism due to hypothyroidism by thyroid hormone (Gordon & Southren 1977) suggest its direct role in reproduction. A recent report from our laboratory has shown that occupation of rat granulosa cell nuclear tri-iodothyronine (T_3) receptor by T_3 induces the generation of a 53 kDa putative protein, T_3-induced protein (TIP) which, in turn, stimulates progesterone (P_4) release (Bandyopadhyay *et al.* 1996). TIP has also been purified from piscine ovarian follicles (Bhattacharya *et al.* 1996) and goat testicular Leydig cells (Jana *et al.* 1996); in both cases it stimulated steroid hormone release. We have earlier reported nuclear T_3 receptor in human luteal cells (Bhattacharya *et al.* 1988) and in this communication we present evidence that T_3 stimulation of P_4 release from human luteal cells is mediated by a proteinaceous factor. Heterologous TIPs also greatly increased P_4 release from luteal cells.

Materials and Methods

Collection of blood from euthyroid and hypothyroid women

Medical practitioners in the vicinity of our university and the university medical hospital always refer patients to our laboratory whenever they suspect thyroid problems. For the present investigation we have requested that donors give blood samples during the mid-luteal phase (days 18–23) of their menstrual cycles. Although some of the hypothyroid patients reported irregular menstruation or bleeding problems, there was no problem in identifying the duration of the luteal phase. We have obtained co-operation from 29 women donors aged between 19 and 35 years, 17 of them were euthyroid and 12 were hypothyroid. Blood (2·0 ml) was collected in a heparinised vial and centrifuged to collect the plasma.

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Human luteal cells

Human corpora lutea were obtained from the ovaries of euthyroid patients undergoing laparotomy for non-endocrine conditions during the mid-luteal phase of their menstrual cycles (days 18–23 of the cycle). The age of the patients was between 32 and 37 years and the indications for surgery were menorrhagia due to fibroids. The process had the approval of the local hospital (Suri Sadar Hospital, West Bengal, India) from where a portion of the ovary containing the corpus luteum was obtained. The ovaries were transported to the laboratory in ice-cold culture medium (Medium 199 with Hank's salt; GIBCO, New York, NY, USA) and all the incubations were conducted within two hours after surgery. We obtained a total of nine ovaries containing the corpus luteum. The corpus luteum was dissected out and placed in chilled culture medium that was previously gassed with 95%O_2/5%CO_2. The luteal portion of the tissue was separated with scissors and forceps and cut into small pieces. Small pieces of tissue were subjected to 0·05% collagenase (collagenase Type II; Sigma Chemical Co., St Louis, MO, USA) treatment for 90 min. Cells were harvested, washed twice and resuspended in the same medium. After counting, the cell suspension was diluted with the medium and 100 µl aliquot (containing 1×10^5 cells) was added to each well in a microwell module (F-16, capacity 400 µl; NUNC, Roskilde, Denmark). Viability of the cells was examined by the trypan blue (0·1%) dye exclusion method which showed approximately 90% viability. The functional ability of the isolated luteal cells was examined by adding ovine luteinizing hormone (oLH, lot no. APF-7071 B; NIDDK, Bethesda, MD, USA) to each well containing 1×10^5 cells. The rest of the cells were kept on ice for further experiments and used only when they were found to respond to oLH. If the luteal cells released significant amounts of P_4 and 17β-oestradiol (E_2) into the medium (estimated by RIA, Mukherjee *et al.* 1994) in response to oLH (Fig. 1), the rest of the cells were used for experiments with T_3, TIP, thyroid hormone-induced factor (TIF), etc. This incubation to examine the functional ability of luteal cells was for 45 min. The interassay and intra-assay coefficients of variation were <10% and 5% respectively for P_4 and <12% and 7% respectively for E_2.

Experiments

Each experiment was conducted with luteal cells collected from one human ovary supplied by the Suri Sadar Hospital. Hence, when five experiments/observations are mentioned, it means luteal cells obtained from five different ovaries containing corpora lutea. One experiment/observation with luteal cells was performed by collecting cells from one ovary, diluting with the culture medium so that 100 µl contained 1×10^5 cells and adding this to each well for incubation with T_3 or TIP or TIF etc. Each treatment was in duplicate, the mean value of duplicate incubations was taken and such mean values from four, five or six different experiments were subjected to statistical analysis. Hence, when four, five or six experiments/observations are mentioned in the legends of tables and figures, it means the data from luteal cell incubations from four, five or six separate ovaries.

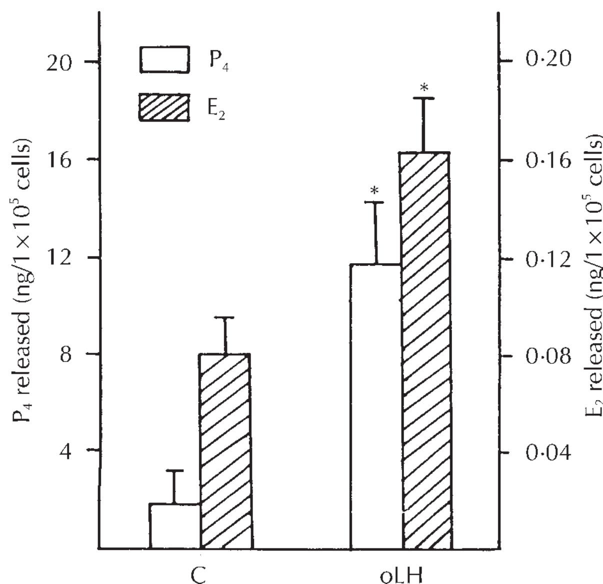

Figure 1 Effect of ovine luteinising hormone (oLH, 250 ng/ml) on progesterone (P_4) and oestradiol (E_2) release from human luteal cells. Cells were incubated either in the absence (control, C) or presence of oLH for 45 min. Values are means ± S.E.M. of nine experiments with cells from nine corpora lutea. *$P<0·05$ compared with control.

Incubation of cells

Luteal cells were incubated in an atmosphere of 95%O_2/5%CO_2 at 37 °C with gentle shaking for 5 h. At the end of 2 h, T_3 (125 ng/ml; dissolved with the help of 0·01 M NaOH) or T_3 plus cycloheximide (Chx, 50 µg/ml) or purified TIP (T_3-induced protein, 5 µg/ml) from rat granulosa cells (rTIP) (Bandyopadhyay *et al.* 1996) or fish ovarian follicles (fTIP) (Bhattacharya *et al.* 1996) were added to the well. A 2-h preincubation time was required for the cell to recover from the shock of separation. Some incubations were carried out with an anti-T_3 antibody (diluted 1:1000 and 50 µl added to the well giving a final dilution of 1:8000; Bhabha Atomic Research Center, Trombay, Bombay, India). The total volume of each incubation was 400 µl.

Purification of TIP and TIF

Purification of fTIP and rTIP was reported earlier (Bhattacharya *et al.* 1996, Bandyopadhyay *et al.* 1996).

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Briefly, either fish ovarian follicles or rat granulosa cells were lysed by sonication after T_3 treatment followed by ultracentrifugation at 100 000 ***g***, and the supernatant was collected and dialysed against distilled water at 4–7 °C for 6 h. Dialysed material was subjected to gel permeation chromatography, peak II of which was chromatographed through FPLC Mono Q and the TIP active protein peak was further purified by FPLC Superose 6 chromatography. At each step of purification, fTIP activity i.e. the ability of pooled fractions to stimulate the conversion of [^{3}H]pregnenolone to [^{3}H]progesterone was assessed. Protein from each peak (pooled fractions) was added to separate ovarian follicle incubation in the presence of [^{3}H]pregnenolone; on termination of incubation, ovarian follicles were homogenised by sonication and the amount of [^{3}H]progesterone formed from the precursor was determined by TLC (Bhattacharya *et al.* 1996). In the case of rTIP purification, TIP activity was determined by adding an aliquot from the protein peaks to rat granulosa cell incubation and the amount of P_4 released into the medium was estimated by RIA (Bandyopadhyay *et al.* 1996). The molecular mass of rTIP was 53 kDa (Bandyopadhyay *et al.* 1996) and that of fTIP was 52 kDa (Bhattacharya et al. 1996).

A partially purified preparation of TIP, termed TIF (T_3-induced factor), from human luteal cells was added to the incubation to compare its effect with purified TIPs. TIF was prepared by incubating human luteal cells with T_3 (125 ng/ml) for three hours. On terminating the incubation, cells were lysed by sonication (132 kHz, Labsonic 2000, B Braun, Germany) followed by centrifugation at 1000 ***g***. The pellet (containing cell membranes and nuclear membranes) was discarded and the supernatant was recentrifuged at 10 000 ***g*** for 15 min; the supernatant of this separation was finally centrifuged at 100 000 ***g*** in a Beckman (model L7–55, Beckman Instruments Inc., Palo Alto, CA, USA) ultracentrifuge for 1 h. The 100 000 ***g*** supernatant (100 K supernatant) was then dialysed (Slide-A-Lyzer Dialysis Cassettes, Pierce Chemical Company, Rockford, IL, USA; molecular exclusion limit: 10 K) against distilled water for 6 h at 4–7 °C. Dialysed material was chromatographed on a Sephadex G 100 (Pharmacia, S-75182, Uppsala, Sweden) column, and the second peak (PII) was collected by a similar procedure as described earlier (Bandyopadhyay *et al.* 1996, Bhattacharya *et al.* 1996, Jana *et al.* 1996). This PII which had TIF activity, was again dialysed so that it was completely free of T_3 contamination. T_3 RIA of the dialysed fraction showed no trace of T_3. A control 100 K supernatant from cells incubated without T_3 and also chromatographed on Sephadex G 100, did not show PII, but fractions of the same zone were pooled and used for comparison (this is referred to as SG-100 F). Ten microgrammes protein of TIF or SG-100 F (from control cells) were added to the incubations. The total volume of each well was 400 µl; hence the concentration of TIF or SG-100 F would be 25 µg/ml.

To confirm the proteinaceous nature of TIF, it was subjected to heat or trypsin treatment prior to its addition into the cell incubation. TIF (10 µg) was added to trypsin solution (200 µg trypsin/ml; Sigma Chemical Co.) with 0·05 M phosphate buffer, pH 7·6, and incubated for 90 min; the incubation was terminated by adding 200 µg/ml soybean trypsin inhibitor (Sigma Chemical Co.). Heat treatment was carried out by taking 10 µg TIF in 100 µl phosphate buffer (0·05 M, pH 7·6) in a glass test tube and boiling in a water bath for 10 min. It was cooled and added to the luteal cell incubations. In all cases, P_4 released into the medium was estimated by RIA (described below).

Protein synthesis in luteal cells

Protein synthesis in human corpora luteal cells in the absence (control) or presence of T_3 (125 ng/ml) was determined by following the procedure described earlier (Jana & Bhattacharya 1994, Bandyopadhyay *et al.* 1996). Briefly, cells were incubated with [^{14}C]leucine (specific activity 300 mCi/mmol; obtained from Bhabha Atomic Research Center) plus 1 mM of 18 different amino acids except leucine. After two hours preincubation, the cells were incubated for three hours in the presence of T_3 (125 ng/ml) or T_3 with Chx (50 µg/ml) or in the absence of these substances (control). At the end of the incubation, the cells were harvested by centrifugation at 500 ***g*** for 10 min, resuspended in distilled water and subjected to ultrasonication (132 kHz). The sonicated material was precipitated with a final concentration of 10% trichloroacetic acid (TCA). The pellet obtained after centrifugation at 3000 ***g*** was washed with 10% TCA and subsequently with 5% TCA containing cold leucine (1 mM). 7% TCA was added to the pellet and the sample was heated for 30 min at 95 °C to denature the nucleic acids. The sample was precipitated by centrifugation at 3000 ***g*** for 10 min and washed twice with 5·0 ml ethanol:ether (1:1). The final precipitate was dissolved in 200 µl NCS (a tissue solubilizer from Amersham International plc., Amersham, Bucks, UK) and counted in a liquid scintillation counter (LS 6000 SC, Beckman) in 10 ml liquid scintillation cocktail (Ready Safe, Beckman). TCA precipitable radioactivity is expressed as d.p.m./mg protein.

RIA of P_4 and thyroid hormones

RIA of P_4 was performed as described previously (Mukherjee *et al.* 1994). Briefly, the P_4 content of the medium (collected after luteal cell incubation) was estimated by incubating it with phosphate-buffered saline (10 mM sodium phosphate pH 7·4 and 0·15 M NaCl) containing 0·1% gelatin, P_4 antiserum (donated by Dr Gordon Niswender, Colorado State University, Colorado, USA) and [^{3}H]P_4 ([1,2,6,7,-^{3}H]progesterone; Amersham International plc). Incubations were carried out for 16 h at 4 °C. Bound and free [^{3}H]P_4 were separated by using

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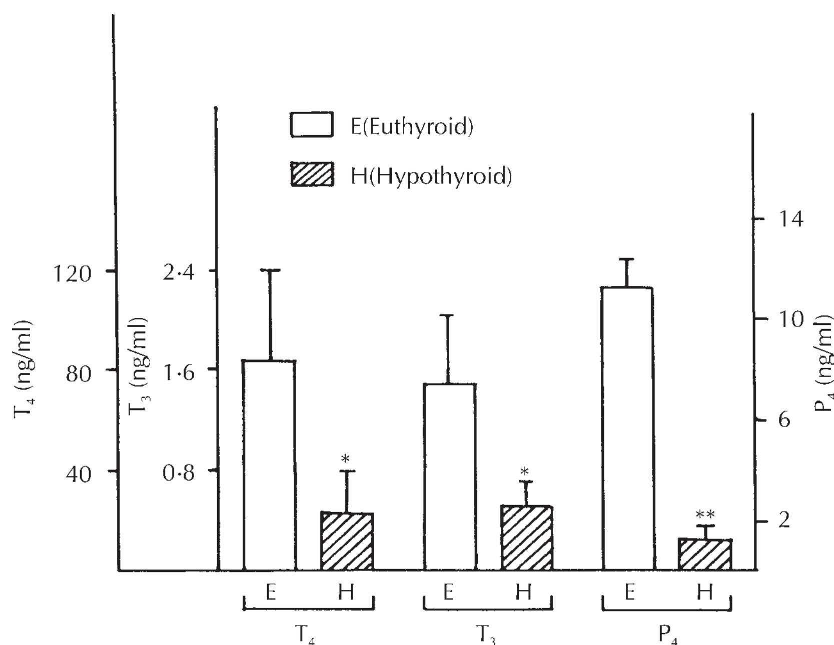

Figure 2 T_4, T_3, and P_4 concentrations in blood samples from women in the luteal phase of the menstrual cycle: n=17 euthyroid women donors and n=12 hypothyroid women donors. See text for details. *P<0·05, **P<0·01 compared with euthyroid donors.

dextran-coated charcoal (0·6% activated charcoal and 0·06% dextran), an aliquot of bound [^{3}H]P_4 was added to the scintillation fluid (Ready Safe) and counted in a liquid scintillation counter. The results are expressed as ng P_4 released per 1 × 10^5 cells (intra-assay and interassay coefficients of variation were <5% and 10% respectively). RIAs of thyroxine (T_4) and 3,5,3′-tri-iodothyronine (T_3) were carried out by utilising the T_4 and T_3 RIA kits from Bhabha Atomic Research Centre (intra-assay and interassay coefficients of variation were <3% and 9% respectively).

Protein estimation

Protein contents of TIP, TIF, SG-100 F and lysed cells (sonicated) were estimated by the method of Bradford (1976) using bovine serum albumin as the standard.

Statistical analysis

Data were analysed by one-way analysis of variance (ANOVA), where the F-value indicated significance. Means were compared by a *post-hoc* multiple range test. All values are expressed as means ± S.E.M.

Results

P_4 level in euthyroid and hypothyroid women

Plasma T_4, T_3 and P_4 levels were estimated in 29 women during their luteal phase; 17 women were euthyroid while 12 were hypothyroid both clinically and also on the basis of their T_4 and T_3 levels. Figure 2 shows that hypothyroid women had significantly lower P_4 levels (P<0·01). The P_4 level in hypothyroid women ranged between 0·2 and 2·2 ng/ml, whereas in euthyroid women it was between 8 and 14 ng/ml. This indicates a relationship between hypothyroidism and low P_4 secretion.

Stimulation of P_4 release from the luteal cells by T_3

Incubation of human corpora luteal cells from euthyroid women in the presence of increasing doses of T_3 caused a dose-dependent stimulation of P_4 release following 25 and 50 ng T_3; 100 ng had no additional effect over the 50 ng dose (Fig. 3). There was a significant (P<0·01) stimulation of P_4 release by 25 ng T_3 in comparison to control, and 50 ng T_3 elevated P_4 release significantly over the 25 ng dose (P<0·05). To substantiate the stimulatory effect of T_3 on luteal cell P_4 release, anti-T_3 antibody was added to the T_3 incubation. Table 1 shows that anti-T_3 antibody reduced the stimulation of P_4 release nearly to the level of the controls indicating specificity of the T_3 stimulatory effect. Addition of Chx also blocked the T_3 stimulatory effect on P_4 release suggesting mediation of the T_3 effect by a proteinaceous factor.

Protein synthesis

Figure 4 shows that T_3 augmented cellular protein synthesis. There was a greater than 3·5-fold increase in

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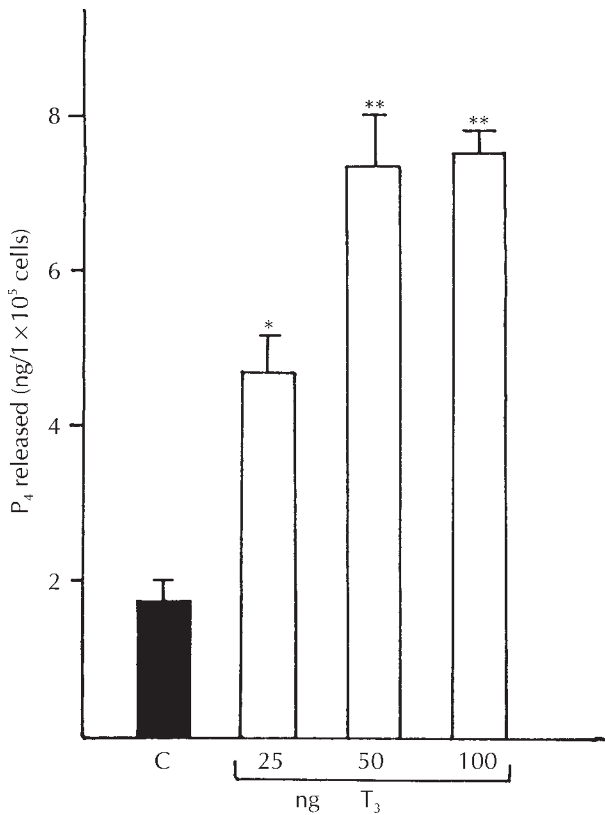

Figure 3 Effect of various doses of T_3 on P_4 release from human luteal cells. Increasing concentrations of T_3 were added to luteal cell incubations and on termination of incubation (3 h), the media were saved for P_4 RIA. T_3 concentrations in this Figure represent the actual amount added to each well (400 µl volume). C, control. Values are means ± S.E.M. of six experiments. *$P<0·01$ compared with control, **$P<0·05$ compared with the effect of 25 ng T_3.

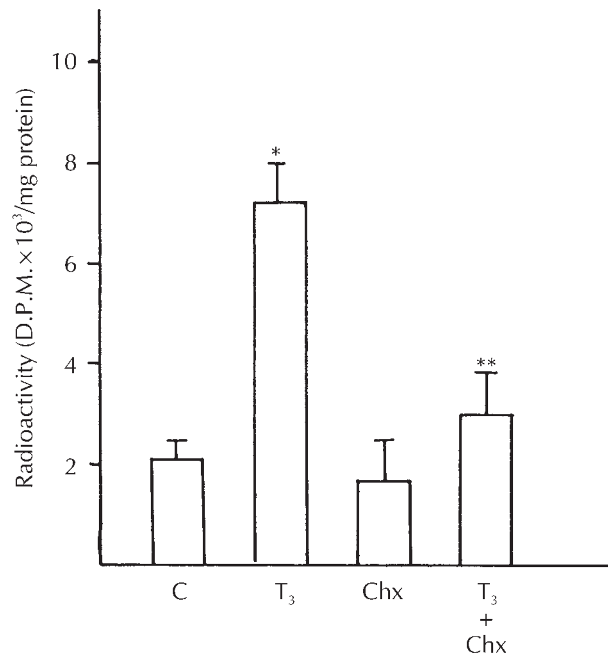

Figure 4 Incubation of human luteal cells with [^{14}C]leucine and 18 other amino acids to determine protein synthesis. Cells were preincubated for 2 h and then incubated for 3 h in the absence (C, control) or presence of T_3 (125 ng/ml) and/or cycloheximide (Chx; 50 µg/ml). Values are means ± S.E.M. of five experiments. *$P<0·01$ compared with control, **$P<0·01$ compared with T_3 incubation.

protein synthesis in response to T_3 compared with control. The increase was inhibited by Chx.

TIP stimulates P_4 release

rTIP or fTIP, TIF and similar fractions from control cells (SG-100 F) were separately incubated with luteal cells.

Table 1 Inhibition of T_3-stimulated P_4 release from human luteal cells by cycloheximide (Chx) or T_3-antibody. T_3 (125 ng/ml), T_3 with anti-T_3 antibody or T_3 with Chx (50 µg/ml) were added to the luteal cell incubation. Values are means ± S.E.M. of six observations

Treatment	**P_4 released** (ng/l × 10^5 cells)
Control	1·81 ± 0·32
T_3	7·32 ± 0·91*
Chx	1·69 ± 0·48
T_3+T_3 antibody	2·61 ± 0·83**
T_3+Chx	2·01 ± 0·75**

*$P<0·01$ compared with control; **$P<0·01$ compared with T_3 alone.

TIF significantly ($P<0·05$) stimulated P_4 release, whereas SG-100 F had no stimulatory effect. There was a remarkable increase in P_4 release ($P<0·01$) in the presence of rTIP or fTIP (Fig. 5). The stimulatory factor in TIF that causes elevation of P_4 release is protein in nature, like rTIP and fTIP, since treatment with trypsin or heat destroyed the stimulatory effect of TIF (Table 2).

Discussion

An earlier report from this laboratory demonstrated the presence of T_3 receptor in the nuclear fraction of human luteal cells (Bhattacharya *et al.* 1988). At that time, it was difficult to speculate on the physiological relevance of this receptor in luteal cells. However, it has been reported that abnormalities in steroid hormone metabolism due to hypothyroidism can be improved by restoring the euthyroid state (Gordon & Southren 1977) and that irregular menstrual bleeding in hypothyroid women is related to low P_4 secretion during the luteal phase (Ingbar & Woebar 1981). In addition to these reports, it is interesting to note that in the current study deficiency in plasma T_3 and T_4 levels in women coincided with a lower circulatory level of P_4. Blood was collected from women subjects during their mid-luteal phase (between days 18 and 23 of the

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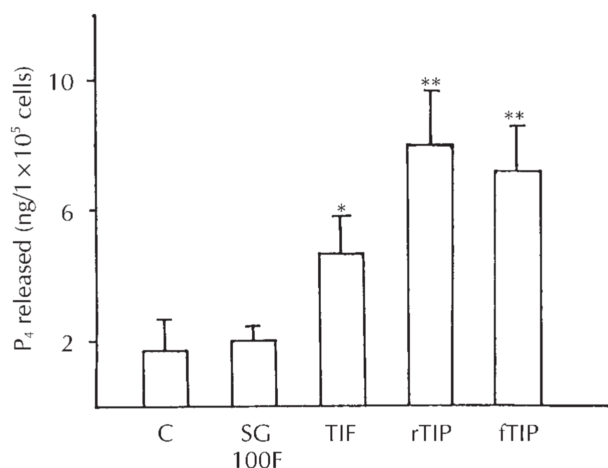

Figure 5 Stimulation of P_4 release from human luteal cells by T_3-stimulated human luteal cell factor (TIF, 25 µg/ml), purified rat granulosa cell T_3-induced protein (rTIP, 5 µg/ml) or purified T_3-induced protein from fish ovarian follicles (fTIP, 5 µg/ml). C, control (incubation of human luteal cells alone); SG-100 F, Sephadex G 100 PII of control cells was added (25 µg/ml) to the luteal cell incubation. Values are means ± S.E.M. of five experiments. *P<0·05, **P<0·01 compared with control.

menstrual cycle). Euthyroid women who had normal plasma T_3 and T_4 levels also showed normal P_4 levels in the luteal phase. These findings indicate thyroid hormone influence on ovarian steroid hormone release and in this context a T_3 receptor in luteal cells appears meaningful.

T_3 significantly stimulated P_4 release from luteal cells collected from the ovaries of euthyroid women. This result confirms our earlier findings with goat Leydig cells (Jana & Bhattacharya 1994), rat granulosa cells (Bandyopadhyay *et al.* 1996) and piscine ovarian follicles (Bhattacharya *et al.* 1996). Failure of normal P_4 secretion in thyroid hormone-deficient women during the luteal stage, the presence of thyroid hormone receptors in human luteal cells and the dose-dependent increase in P_4 release from human luteal cells in response to T_3 all suggest a link between thyroid hormone and P_4 secretion from luteal cells. At this point, a question as to whether the T_3 stimulation of P_4 release is distinct from LH stimulation seems to be very relevant. We have previously shown that T_3 has an additive effect on LH-stimulated androgen release from goat Leydig cells (Jana & Bhattacharya 1994) and P_4 release from rat granulosa cells (Bandyopadhyay *et al.* 1996). These reports suggest that the action of T_3 is distinct from that of LH.

Table 2 Effect of trypsin (200 µg/ml) and heat (100 °C for 10 min) on the ability of TIF (10 µg protein) to stimulate P_4 release from human luteal cells. Values are means ± S.E.M. of six observations

Treatment	**P_4 released** (ng/l × 10^5 cells)
Control	1·75 ± 0·37
TIF	4·85 ± 1·11*
TIF+trypsin	1·62 ± 0·48**
TIF+heat	1·57 ± 0·98**

*P<0·05 compared with control; **P<0·05 compared with TIF alone.

T_3-stimulated P_4 release from luteal cells can be blocked by Chx, indicating mediation of the T_3 effect by a proteinaceous factor. T_3 also stimulates luteal cell protein synthesis by more than 3·5-fold over the control suggesting the possibility of the generation of a proteinaceous factor which mediates the T_3 stimulatory effect on P_4 release. We have already isolated and purified this factor (i.e. TIP) from goat testicular Leydig cells (Jana *et al.* 1996), rat granulosa cells (Bandyopadhyay *et al.* 1996) and fish ovarian follicles (Bhattacharya *et al.* 1996). Incubation of fish ovarian follicles or rat granulosa cells with T_3 resulted in an approximately 3·5-fold stimulation of protein synthesis which includes other proteins besides TIP. SDS-PAGE of a partially purified fraction (Sephadex G 100 gel filtration) showed three bands containing detectable radioactive counts (when cells were incubated with [^{14}C]leucine and 18 other amino acids in the presence of T_3). This suggests that T_3 induces the synthesis not only of TIP but also of other proteins (Jana *et al.* 1996). Purified TIP in all these cases consists of a single polypeptide chain and has comparable molecular mass. Molecular masses of different TIPs are very close; fish oocyte TIP is a 52 kDa (Bhattacharya *et al.* 1996), goat Leydig cell TIP is a 52 kDa (Jana *et al.* 1996) and rat granulosa cell TIP is a 53 kDa (Bandyopadhyay *et al.* 1996) monomer protein. The addition of goat TIP to goat Leydig cells causes stimulation of androgen release. Rat TIP added to rat granulosa cells or fish TIP added to fish oocytes increased P_4 formation and release. Although TIP is a 52/53 kDa protein, it is quickly internalised into the cell (Bhattacharya *et al.* 1996, Bandyopadyay *et al.* 1996). All these data suggest that TIP is a secreted protein that acts in an autocrine manner. We have raised anti-TIP antibody against fTIP which crossreacts with rTIP and Leydig cell TIP. Anti-TIP was used to detect TIP in the rat granulosa cell incubation medium which showed that TIP is a secreted protein (A Bandyopadhyay, P Roy & S Bhattacharya, unpublished observations).

Hence, it was of interest to see whether the partially purified TIF from human luteal cells incubated with T_3 could stimulate P_4 release from luteal cells and whether heterologous TIP has a similar effect on these cells. TIF significantly stimulated P_4 release and its stimulatory effect could be destroyed by trypsin and heat, suggesting its proteinaceous nature. Purified TIP from rat granulosa cells and fish ovarian follicles greatly increased P_4 release from human luteal cells, indicating lack of species specificity in TIP activity. All of them have effects on the stimulation of steroid hormone release. Immunologically, fish anti-TIP antibody crossreacts with other TIPs (P Roy, M Datta & S Battacharya, unpublished observations). All these

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indicate that TIP is an evolutionary conserved protein, but more information on the molecular structure of TIP is necessary before such a conclusion can be made.

Results obtained with human luteal cells in the current experiments yield one important revelation: the nature of thyroid hormone action on these cells is similar to that which has been observed with rat ovarian granulosa cells (Bandyopadhyay *et al.* 1996), goat testicular Leydig cells (Jana & Bhattacharya 1994) and fish ovarian follicles (Bhattacharya *et al.* 1996). In all these cases T_3 stimulation of steroid hormone secretion is not direct; T_3 occupation of receptor results in the synthesis of a novel putative protein, i.e. TIP or TIF which, in turn, stimulates steroid hormone release. Due to the difficulty in obtaining human luteal cells, TIF could not be purified to homogeneity, but its effect is similar to that of purified TIP obtained from other sources and it is also proteinaceous in nature. How TIP stimulates P_4 release is not known; it may elicit $\Delta 5$ 3β-hydroxysteroid dehydrogenase activity as observed in fish ovarian follicles (Bhattacharya *et al.* 1996). This aspect is important and requires further study. The reason for reproductive malfunction due to hypothyroidism is not known, but findings described in this communication clearly suggest a pathway of thyroid hormone influence on gonadal function.

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