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Thyroid hormone binds to human corpus luteum

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Summary. A high affinity, low capacity 3,5,3'-triiodo-L-thyronine (T_3) specific binding was detected in the nuclei preparation from human corpus luteal nuclei by using an in vitro thyroid hormone binding assay. The bound hormone was efficiently separated from free hormone by the use of 40% polyethylene glycol. Under standardized assay conditions of pH 8.6 at 37 °C and a 2-h incubation time, the binding in the corpus luteal nuclei was saturable with Kd 4.94×10^{-10} M with low maximum binding capacity (1.70 p mol/mg DNA). Competitive binding studies with hormone analogues indicated that T_3 binding to corpus luteal nuclei is hormone-specific. Findings indicated a direct effect of thyroid hormone on the human corpus luteum.
Key words. Thyroid hormone; human corpus luteum; triiodothyronine binding.

Thyroid has long been involved in the reproduction of vertebrates[1-7]. Thyroid hormone has been found to influence both ovarian[8,9] and testicular[10-13] functions in mammals. Hypothyroid women have irregular menstrual bleeding due to the failure in progesterone secretion[14]. Alteration in steroid hormone metabolism due to hypothyroidism can be improved by restoring the euthyroid state[15]. Although these reports suggest an influence of thyroid hormone on gonadal activity, how it does so remains unclear. Recently, we have reported high affinity and low capacity thyroid hormone binding sites in the ovarian nuclei of perch[16] implying a direct involvement of thyroid hormone in the reproduction. Thus it would be worthwhile to evaluate reproductive malfunction in hypothyroid patients provided binding sites for thyroid hormone can be detected in human ovaries. We describe here the validation of an in vitro triiodothyronine (T_3) binding assay for human corpus luteum nuclei and examine the binding activity of different thyroid hormone analogues.

Materials and methods. The minute portion of the ovary containing corpus luteum was obtained from women undergoing laparatomy for nonendocrine conditions during the luteal phase of the menstrual cycle. The average age was between 37–40 years. The procedure had the approval of the local hospital (Suri Sadar Hospital, West Bengal, India). Indications for surgery were as follows: a) menorrhagia, b) menorrhagia in fibroids, c) fibroids, d) carcinoma of the cervix and e) sterility.

Nuclei from corpus luteal cells were isolated following the method of Jackson and Chalkey[17] as described by Lawson et al.[18]. All isolation procedures were carried out near 0 °C. The corpus luteal tissue, after removal, was immediately kept in ice-cold SMNaT buffer (0.25 M sucrose, 10 mM $MgCl_2$, 50 mM $NaHSO_3$ in 10 mM Tris buffer, pH 7.5) and washed thoroughly. It was then cleaned, blotted, weighed, minced and homogenized in SMNaT buffer containing 1% (v/v) Triton X-100 using a Potter-Elvehjem type glass homogenizer. The homogenate was passed through double-layered cheese cloth and centrifuged at 2000 g in a refrigerated centrifuge. The pellet containing the crude nuclei was rehomogenized and washed thrice as above. Finally the pellet was washed twice in SMNaT buffer (without Triton X-100) to remove Triton X-100. At each step the nuclei were microscopically checked (Carl-zeiss, Jena) with aceto-orcein stain (2% orcein in 50% glacial acetic acid). The final nuclear material was found to be free of 'ghost' cell membranes or other debris and was designated as 'pure nuclear preparation'. This preparation was immediately used for receptor binding study, although it is stable at −20 °C for more than 7 days.

In vitro T_3-binding assay was based on the description of DeGroot and Torresani[19] and Darling et al.[20] with a few modifications reported earlier by us[16]. Standardization of T_3 binding assay for luteal cell nuclei preparation include optima for time, temperature, pH and polyethylene glycol (PEG) concentration, and these are described in the results. For Scatchard analysis, nuclear preparation was suspended in SMCT incubation buffer (binding assay buffer) containing 0.32 M sucrose, 3 mM $MgCl_2$, 2 mM $CaCl_2$, 5 mM DTT in 10 mM Tris buffer, pH 8.6. 80 µg of DNA (per tube) was incubated with increasing concentrations of ^{125}I-T_3 (sp. activity 180.6 µCi/µg) from 2.833 to 0.194 p mol in 500 µl of SMCT buffer at 37 °C for 2 h. DNA was estimated following the procedure of Howell[21] and also by spectral analysis as mentioned by Lawson et al.[18]. Both methods gave similar results. The amount of nuclei added in each incubation was determined by the quantity of DNA added to each tube. The incubation was terminated by placing the tubes on crushed ice for 10 min. Bound and free hormones were separated by adding chilled 40% PEG (mol. wt 6000). The tubes were kept in ice for 10 min and then centrifuged at 2500 g. Supernatant was aspirated and the pellet was counted in a Gamma Counter (ECIL, India). Nonspecific binding was determined

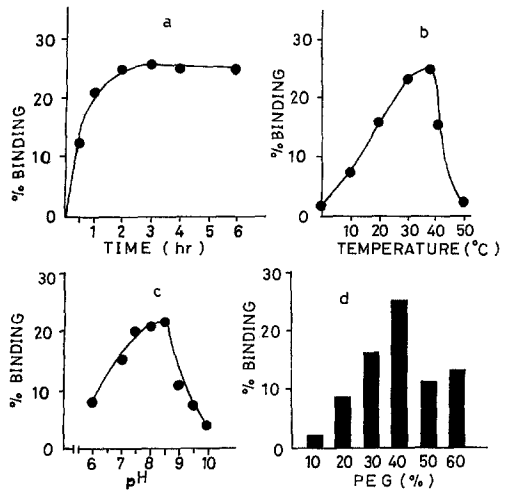

Figure 1. The specific binding of T_3 to human corpus luteal nuclei at various times (*a*), temperatures (*b*), pH levels (*c*) and PEG concentrations (*d*). The data represent the mean of five observations.

by incubating the same amount of radiolabeled T_3 with 400 p mol of unlabeled T_3. For the displacement study the above-mentioned parameters were kept unchanged except the use of analogues in varied concentrations.

Results and discussion. T_3 binding to luteal cell nuclei was half maximal at 30 min and maximal at 2 h. The binding plateau was maintained for 6 h, indicating saturable binding sites for T_3 (fig. 1 a). Binding of T_3 was very sensitive to pH and temperature. Maximum specific binding occurred at 37 °C and at pH 8.6 (fig. 1 b and c). On either side of temperature and pH optima, there was a sharp decline in binding activity. After separating bound T_3 from free T_3 by centrifugation of nuclei, it was found that the free fraction retained some specifically bound hormone. This was evident by the use of activated charcoal or PEG (data not shown). The results obtained with varied concentrations of PEG showed that addition of 40% PEG greatly increased the specific binding (fig. 1 d). Use of 40% PEG completely precipitated T_3 binding protein which was confirmed by adding activated charcoal to the aliquot. This supports the contention of Darling et al.[20], and our earlier findings with perch ovarian nuclei[16], that T_3 binding protein released from the nuclei during the incubation at 37 °C is precipitated by PEG along with bound hormone. Bernal and DeGroot[22] reported that rat hepatic nuclei lose putative T_3 receptors from chromatin protein during in vitro incubation. This problem of loss of T_3 binding protein from nuclei can be efficiently solved by the use of optimum concentration of PEG.

Affinity constant was determined from saturation experiments, where 80 µg DNA was incubated with increasing concentrations of ^{125}I-T_3, from 2.833 to 0.194 p mol. Scatchard analysis of the data clearly exhibited high affinity and low capacity binding sites for luteal cell nuclei (fig. 2). The Kd was 4.9×10^{-10} M and maximum binding capacity (MBC) was 1.70 p mol/mg DNA. This shows that binding of T_3 to luteal cell nuclei is saturable with high affinity and low capacity. Nuclei binding of T_3 has been reported in rat liver and kidney[23], in rat heart, brain, spleen and testis[24], in human liver[25] and in rat uterus[26], and this laboratory[16] was the first to report binding of T_3 to ovarian nuclei. T_3 binding to perch ovarian nuclei had a much lower affinity (Kd = 9.1×10^{-9} M) as compared to that of the human corpus luteal nuclei. Again T_3 binding capacity was considerably higher in perch ovarian nuclei (MBC = 4.312 p mol/mg DNA). Binding affinity of T_3 to human corpus luteal nuclei is comparable to that with rat uterine nuclei[26] although Kd value of rat uterine nuclei was a little higher (Kd = 6.1×10^{-10} M) indicating higher binding affinity of luteal nuclei. Competitive binding experiments were carried out at optimal assay conditions with variations in T_3, Triac and T_3 concentrations. Results clearly show (fig. 3) that T_3 binding sites in luteal cell nuclei are analogue-specific. T_4 is a poor competitor while Triac binds with almost equal affinity. Relative binding affinities examined in this system are very similar to those reported with hepatic nuclei[19, 20, 27, 28]. A hormone receptor is characterized by its saturability, high affinity and low capacity, and analogue specificity in target tissues. T_3 binding to human corpus luteal nuclei has these characteristics indicating that it represents T_3 receptor activ-

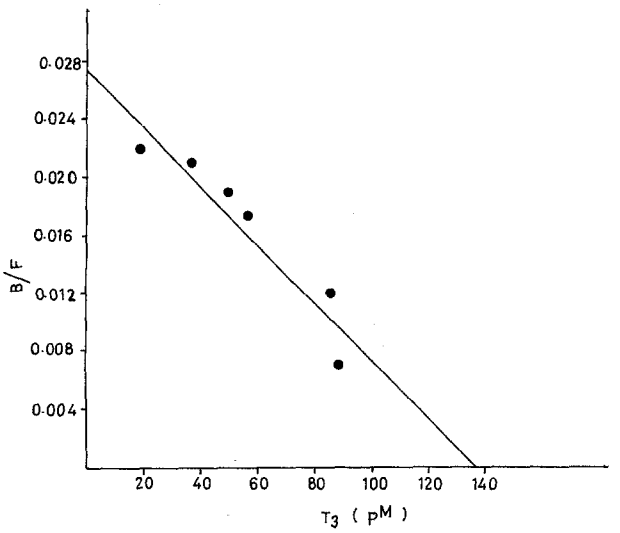

Figure 2. Scatchard plot of T_3 binding to corpus luteal nuclei. The X axis represents the picomolar concentration of bound T_3 and Y axis represents bound to free ratio (B/F). Each point in the plot represents mean of five observations.

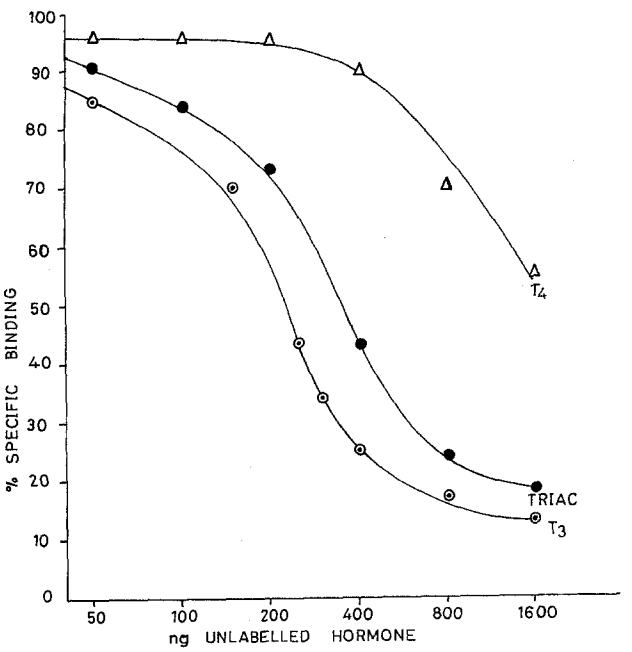

Figure 3. Competitive inhibition of radiolabeled T_3 binding to corpus luteal nuclei by Triac, T_4 and T_3. Each point on the curve is the mean of four determinations.

ity. This suggests that thyroid hormone is directly involved in human ovarian function but the precise mechanism behind its involvement still remains to be elucidated.

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Vitamins and other metabolites in various sera commonly used for cell culturing

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Summary. Many cell culture media use different sera to enhance growth. We assayed vitamins and some related metabolites in different sera and identified the concentration of: thiamin, biotin, folates, riboflavin, pantothenates, nicotinates, vitamins B_6, B_{12}, A, E, C, and carotenes and some related metabolites: biopterins, free inositol, free and total choline, total carnitines in chicken, horse, rabbit, goat, pig, calf, newborn calf, fetal calf and human sera. Results indicate that vitamin and metabolite content of different sera vary. Such variations could produce fluctuant effects on cell culturings if the metabolite content of the serum is not documented.
Key words. Vitamins; cell culture.

Nearly all cell culture media have additions of serum, ascitic fluid, tissue extracts or the like for enhanced growth[1-4]. Cultured cells in defined or semi-defined media generally contain choline, folic acid, nicotinamide, pantothenate, pyridoxal, thiamin, cyanocobalamin and inositol[3]. Their concentrations in such media are in many instances very low, hence a quantitation of actual vitamin requirements may be obscured by crude supplements commonly employed. Additional vitamins, for example, are usually contributed by serum additions which moreover contribute various growth stimulants for different cells[1-4]. Because of difficulties in identification of significant growth stimulators in serum, defined media are being devised so constituents are kept as low as practicable[2,3]. Although defined media containing salts, amino acids, purines, pyrimidine and some vitamins provide excellent growth for many cell lines, sustained growth for many lines however require that the medium be fortified with an animal or human serum or some equivalent[3,5,6]; many kinds of sera are available for the purpose. However concentrations of vitamins and other metabolites contained in commercially available sera have, to our knowledge, been scantily documented. Such information could guide the investigator's choice of a serum for studying specific cellular metabolic pathways and growth patterns and may avoid confounding results obtained in cell culturings because of metabolite imbalances. Consequently we assayed vitamins and some related metabolites in various animal and human sera.

Materials and methods. Each of four different pooled batches of pathogen, antibiotic and drug-free serum from: chickens, horses, rabbits, goats, pigs, calves, newborn calves, fetal calves and humans were assayed for vitamins and several metabolites (vide infra). Except for human serum, all other sera were from Sigma Chemical Co. (St. Louis, MO) and