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TRIIODOTHYRONINE DECREASES THE PRODUCTION OF ANDROGEN BINDING PROTEIN BY RAT SERTOLI CELLS

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SUMMARY: Triiodothyronine (T_3) effects on cultured Sertoli cells from immature rats were investigated by evaluating the production of androgen binding protein (ABP) a biochemical marker of Sertoli cell function.
The results demonstrate that T_3 administration to the rat as well as T_3 addition to the culture medium specifically decreases ABP production by Sertoli cells, suggesting a direct regulatory role of thyroid hormone on male reproductive function.

Thyroid hormones have a profound effect on the growth, development and metabolism of practically all tissues of higher organisms. It has been reported that changes in thyroid hormone levels result in male reproductive abnormalities in man and animals (1-4). Thyroidectomy or thyroxine injection also affect the structural and functional testicular activity in the mature rat through an alteration of blood ICSH level (5). Moreover, the fall of testicular weight was demonstrated following thyroidectomy in the adult rat (6). It is not clear however, whether thyroid hormones affect the testis function directly since a response to thyroid hormone was not observed in rat testis, at least using the conventional criterion of the increase in oxygen consumption (7). In addition, the level of the nuclear receptors for thyroid hormones, which have been detected in all tissues that are known to respond to thyroid hormone, is very low in the testis (8).

Recently, it has been reported that prepubertal spermatogenic cells in immature rats show a pronounced dependency on thyroid hormones (9) but no data are available at present, regarding thyroid hormone effects on the metabolism of the Sertoli cells, which represent a very important site of control of the spermatogenic process.

In the present study, triiodothyronine (T_3) effects on Sertoli cells from immature rats are investigated by evaluating the production of androgen bind-

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ing protein (ABP), which is a Sertoli cell specific secretory protein that has been widely used as a biochemical marker of Sertoli cell function (10). The data presented here demonstrate that T_3 specifically decreases ABP production by Sertoli cells and represent the first evidence of a direct action of thyroid hormone on testis function.

MATERIALS AND METHODS

Cell preparation. Male Wistar rats were obtained from Nossan (Correzzana, MI, Italy). Treatment with T_3 (10 μg/100 g weight) was carried out from 20 to 28 days of age by daily intraperitoneal injection of the hormone. Sertoli cell enriched cultures were prepared from either T_3-treated and control rats (28-day-old animals) by a slight modification of the method of Dorrington et al. (11). Seminiferous epithelium explants were cultured at 32°C in Eagle's minimum essential medium (MEM) buffered with 20 mM HEPES, pH 7.4 and supplemented with 0.1 mM non essential amino acids and 50 U/ml penicillin-streptomycin solution. After 24 hr of culture the medium was replaced by fresh medium with or without 10^{-6} M T_3 (Sigma Chem. Co., St. Louis, MO, U.S.A.) and/or FSH (5 μg/ml) supplied by the NIADDK, National Hormone and Pituitary Program.

Assay of medium ABP and lactate. Culture media from Sertoli cell enriched cultures were collected, centrifuged to remove debris, aliquoted and frozen. Medium ABP concentration was assayed by equilibrium dialysis against 1 nM [^{3}H] dihydrotestosterone (55.5 Ci/mmol, NEN, Dreieich, W. Germany) as described by Fritz et al. (12). The lactate concentration was determined UV-enzymatically according to Noll (13).

Protein turnover and secretion. To evaluate the time course of protein labelling, Sertoli cell monolayers were incubated for 1.5 and 3 hr at 32°C in 2 ml of MEM containing 0.3 mCi/ml [^{14}C]valine (NEN). After incubation cells were rinsed twice with cold saline, scraped from the dishes and precipitated in 10% TCA. The precipitate was assayed for incorporated radioactivity by liquid scintillation counting (14); the trichloroacetic acid extract was used for the determination of intracellular [^{14}C]valine radioactivity and for the analysis, by HPLC (15), of intracellular valine pool. The specific activity of cellular valine was calculated by dividing the acid soluble radioactivity by valine concentration. Newly synthesized secreted proteins were evaluated by measuring the radioactivity of the extracellular acid insoluble fraction. Protein degradation was measured as the release of [^{14}C]valine from labelled proteins (14); after a 20-hr incubation period with a tracer dose of [^{14}C] valine cell monolayers were rinsed twice with cold saline and then reincubated for 1.5 and 3 hr at 32°C in fresh medium containing a molar excess of unlabelled valine. The release of acid soluble radioactivity in the medium was expressed as a percentage of the total initial protein radioactivity of the cell sample.

Protein content was determined by the method of Hartree (16), using bovine serum albumin as a standard.

Statistical analysis was performed using the Student's t test for paired data.

RESULTS AND DISCUSSION

ABP and lactate production by Sertoli cells obtained from T_3-treated and control rats are shown in fig. 1. It can be seen that T_3 treatment signifi-

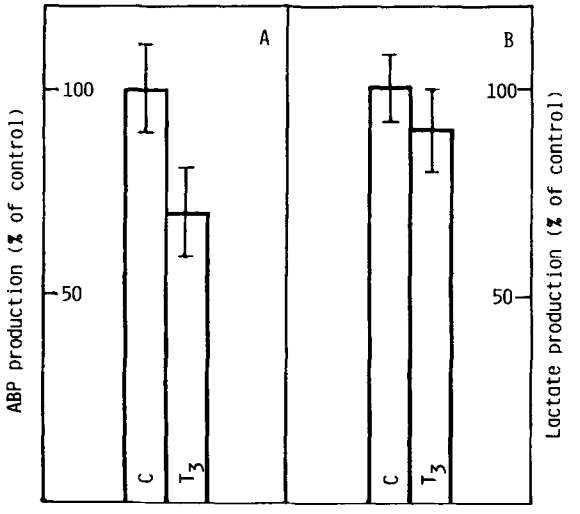

Figure 1. ABP and lactate production by Sertoli cells from T_3-treated and control rats during the initial 24 hr of culture; results, expressed as a percentage of control values, represent the mean ± S.E. of triplicate cell samples from two separate experiments.

cantly lowers the secretion of ABP into the medium of Sertoli cells cultured for 24 hr (panel A). It has been shown that ABP is synthesized "de novo" in cultured Sertoli cells (17) and it is almost completely found in the medium and not associated with the cells. Therefore, the rate of secretion reflects the synthesis of this protein.

The secretion of lactate, used to gain insight into possible T_3 induced changes in Sertoli cell secretory activity, is not affected by the treatment with the hormone (panel B).

ABP and lactate production by Sertoli cells from control rats, cultured in chemically defined medium in the presence or absence of 10^{-6}M T_3 and/or FSH is shown in fig. 2. It can be seen that T_3 addition to the culture medium significantly depresses ABP production by Sertoli cells, whereas the supplementation with FSH stimulates the production of this protein (panel A), as previously reported by others (12). Interestingly, this latter response of Sertoli cells to FSH is antagonized by T_3. Actually, when both the hormones are present in the culture medium the stimulating effect on ABP production by FSH disappears and the inhibitory effect of T_3 prevails. Further investigation is required to ascertain which mechanism(s) is responsible for the opposite effects elicited by T_3 and FSH on ABP production by Sertoli cells.

The addition to the culture medium of lower doses of T_3 (up to 10^{-10}M) resulted in the same inhibitory effect on ABP production (data not shown).

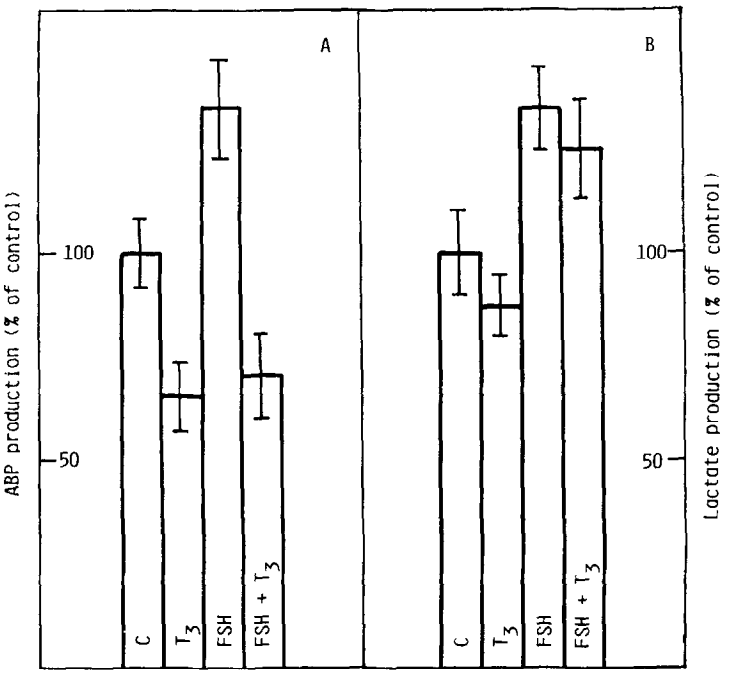

Figure 2. ABP and lactate production by Sertoli cells from control rats cultured in the presence and absence of T_3 and/or FSH. Assays were performed after 24 hr of exposure to the hormones. Results, expressed as a percentage of control values, represent the mean ± S.E. of triplicate cell samples from at least three separate experiments.

Lactate secretion, a reflection of Sertoli cell metabolic activity, does not appear to be significantly affected by T_3 addition to the culture medium, alone or with FSH, whereas the stimulatory effect by FSH has been confirmed in our culture condition (panel B), (18).

To determine whether the T_3-induced decrease in ABP production was due to a generalized decrease in protein synthesis and/or secretion or to an increase in protein degradation, the hormone effect on protein turnover and secretion has been evaluated. With this aim Sertoli cells cultured in the presence or absence of T_3 have been labelled with tracer dose of [^{14}C]valine for 1.5 and 3 hr and then used to measure the incorporation of the radioactive precursor into total intracellular and extracellular proteins. As can be seen in fig. 3, the exposure of Sertoli cells to T_3 does not affect the incorporation of [^{14}C]valine into intracellular nor into extracellular proteins. The extracellular proteins represent newly synthesized proteins secreted into the medium and amount to 20% of total proteins (intra- plus extracellular).

The table 1 shows that T_3 does not affect the overall protein synthesis as

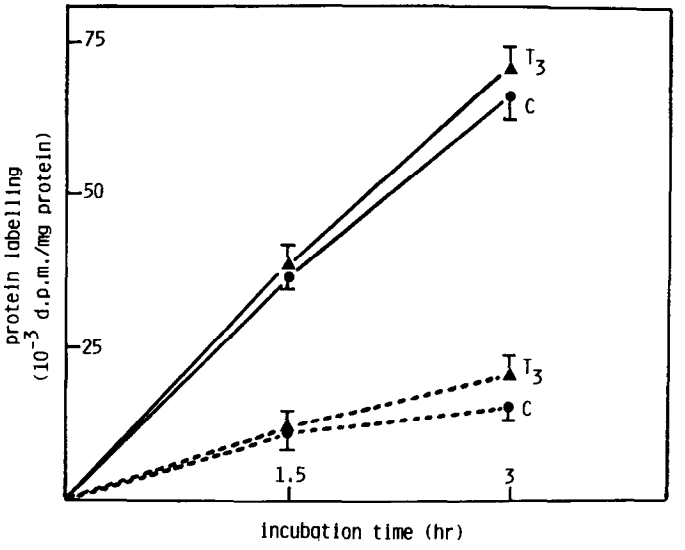

Figure 3. Time-course of protein labelling and secretion in Sertoli cells cultured in the presence or absence of T_3; 24 hr after the hormone was added cells were labelled for 1.5 and 3 hr with tracer dose of [^{14}C]valine and intracellular (——) and extracellular (-----) acid insoluble radioactivity was measured. For details see under Materials and Methods. Each point represents the mean ± S.E. of triplicate plates from at least three separate experiments.

evaluated by dividing the [^{14}C]valine incorporation values by the intracellular specific activity of the labelled precursor.

The time course of protein degradation by Sertoli cells cultured in the presence or absence of T_3 and labelled for 24 hr with tracer dose of [^{14}C] valine is reported in fig. 4. The protein degradation is evaluated as % release of [^{14}C]valine from prelabelled proteins during 1.5 and 3 hr of incubation. As can be seen, the % release of [^{14}C]valine is not affected by T_3.

Table 1. Protein synthesis of Sertoli cells cultured with or without T_3

Culture condition	[^{14}C]valine incorporation (10^{-3} dpm/mg protein)	valine pool s.a. (10^{-3} dpm/nmol)	protein synthesis (nmol/mg protein)
Control	35.3 ± 1.3	3.8	9.2
T_3	36.1 ± 1.7	3.6	10.1

Sertoli cells were labelled for 1.5 hr with a tracer dose of [^{14}C]valine; the radioactivity of acid insoluble material and the specific activity (s.a.) of valine pool were evaluated as described under Materials and Methods. Protein synthesis was determined by the ratio between the radioactivity incorporated per mg of protein and the specific activity of the valine pool. Data represent the mean ± S.E. of triplicate plates obtained from two separate experiments.

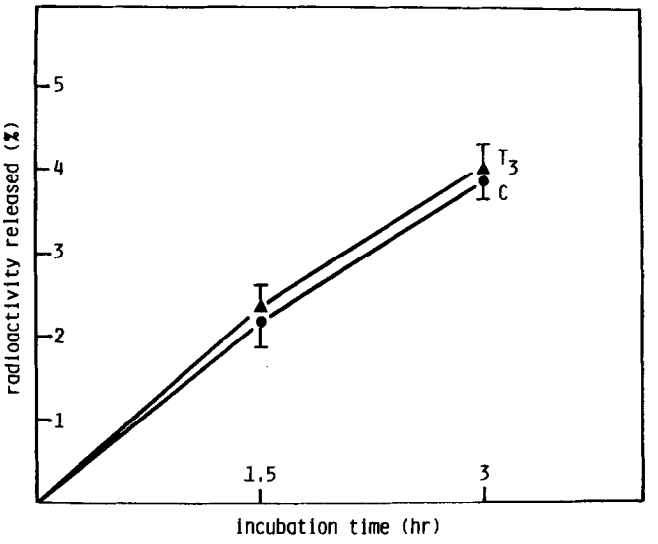

Figure 4. Time-course of $[^{14}C]$valine release from labelled Sertoli cells cultured for 24 hr in the presence or absence of T_3. The radioactivity released at the indicated time is expressed as a percentage of the total protein radioactivity (14). For details see under Materials and Methods. Each point represents the mean ± S.E. of triplicate samples from at least three separate experiments.

On the basis of the evidence that thyroid hormone does not modify the overall protein turnover nor the rate of protein secretion we can conclude that T_3 specifically inhibits the synthesis of ABP.

Taking into account that commercially available serum contains physiological concentrations of thyroid hormone, this inhibitory effect of T_3 could explain the recently reported reduction of ABP production by serum-supplemented Sertoli cells (19).

The use of Sertoli cell culture approach allowed us to demonstrate a specific and direct response to T_3 by a cell population belonging to an organ considered to be unresponsive to thyroid hormone on the basis of conventional criteria (7-8). Our results show that Sertoli cells are a target for T_3 action on the testis, suggesting a possible regulatory role of thyroid hormone on male reproductive function at this level.

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