PERGAMON

Journal of Steroid Biochemistry & Molecular Biology 72 (2000) 133–141

The Journal of Steroid Biochemistry & Molecular Biology

www.elsevier.com/locate/jsbmb

The expression of androgen receptor messenger RNA is regulated by tri-iodothyronine in lizard testis

Anna Cardone[a], Francesco Angelini[a], Teresa Esposito[b], Raffaella Comitato[a], Bruno Varriale[b,*]

[a] *Dipartimento di Biologia Evolutiva e Comparata, Università Federico II, Via Mezzocannone 8, 80134 Napoli, Italy*
[b] *Laboratory of Molecular Biology, Dipartimento di Fisiologia Umana e Funzioni Biologiche Integrate 'F. Bottazzi' II Università di Napoli, Via Costantinopoli 16, 80138 Napoli, Italy*

Received 14 May 1999; accepted 12 November 1999

Abstract

The network of hormonal and non-hormonal signals required for testicular activity during the reproductive cycle of the seasonal breeding lizard, *Podarcis sicula*, are not yet well understood. Androgens are significantly involved in meiosis and spermiogenesis, and such an effect is mediated through their receptor (AR). Estrogens also affect the testicular activity down-regulating the expression of AR mRNA. Since over the last few years, extensive works have reported, in mammals, a clear influence of tri-iodothyronine (T_3), the biologically active thyroid hormone, on Sertoli cell activities, we carried out a study to shead light on the effect/s exerted by T_3 in lizard testis. A thyroid hormone receptor mRNA (TR mRNA) has been found in the testis indicating that T_3 might be involved in the regulation of gonadal activity. In in vivo experiments, injection of T_3 to male lizards, captured during the recrudescence period (March) and maintained under experimental photothermal conditions (24°C and 15 h daylight), increased the expression of AR mRNA. The in vitro results confirmed the stimulatory effect of T_3 on AR mRNA levels. Thus, in testosterone (T) exposed cells, the highest values of AR mRNA were observed in T_3-primed animals, indicating that T and T_3 increase AR gene transcription independently. The present data suggest that, in lizards, the combined action of androgens, estrogen and T_3 might regulate testicular activity, modulating AR mRNA levels.

Keywords: Steroid receptor; Lizard; Reproduction; mRNA expression

1. Introduction

In reptiles, many studies have been conducted to elucidate the dynamic relationships between the elements involved in the control of male gonadal activity, as well as the mechanisms responsible for the action of the regulating factors.

In the seasonal breeding lizard, *Podarcis sicula*, testicular activity is regulated by hormonal signals through mechanisms that are not yet well defined. Like most saurians inhabiting temperate zones, this species displays a reproductive cycle with full gonadal activity in spring and regression in summer. In July, the onset of an endogenous refractory phase blocks spermatogonial mitoses and causes the degeneration of the other germ cells [1–3]. As in other vertebrates, in lizards, specific changes in plasma sex hormone levels occur during the reproductive cycle [4,5] and it has been proposed that androgens are significantly involved in meiosis and spermiogenesis [1,2], and such an effect is mediated through their receptor (AR). Recently, in vitro studies carried out on primary cultures of lizard testis cells during full reproductive activity, have shown that both androgens (T) and estrogens (E) are involved in the regulation of AR mRNA expression [6]. T and E auto-

* Corresponding author. Tel.: +39-081-5665839; fax: +39-081-5665820.

0960-0760/00/$ - see front matter
PII: S0960-0760(00)00021-2

induce the expression of androgen (AR) and estrogen receptor (ER) mRNAs, respectively. Conversely, a marked down-regulatory effect of E on the expression of AR mRNA has been observed [6]. Thus, in lizard testis cells, an interplay between estrogen and androgen receptors occurs.

The regulation of testicular activity, however, depends on several endogenous factors. Many in vivo studies have shown that, in mammals, the thyroid hormone affects testicular growth and differentiation [7–9]. Transient neonatal hypothyroidism in rats causes an increase in testis size [10], sperm production [11] and number of Sertoli cells [12] in adults. These effects in vivo seem to be due to the direct effect of thyroid hormone on the testis [13].

An interaction between thyroid hormone and reproductive activity has long been recognized in other vertebrates. In birds, it has been proposed that thyroxin may contribute to seasonal testicular regression by reducing testis sensitivity to LH [14]. Investigations upon agamids, lacertids and geckonids have shown that hypothyroidism, induced by thyroidectomy, causes a regression of the testis, involving spermatogenetic arrest and reduction in androgen secretion [15,16]. Thyroid treatment stimulates spermatogenesis in *Rana pipiens* [17] and in the toad *Bufo regularis* [18].

Furthermore it has been found that the expression of thyroid hormone receptor (TR) is associated with the expression of other nuclear receptor genes. Thus in *Xenopus* liver the expression of TR potentiates estrogen activation of vitellogenin genes and autoinduction of ER in epatocytes [19]. Also in primary cultures of hamster Harderian gland cells, the expression of AR mRNA is increased when T_3 is present in the culture medium [20]. Since there are now studies regarding the cross-talk between different nuclear receptors in the view of multiple control of some physiological functions such as testicular activity, the aim of the present study was to determine if T_3 might be involved in the regulation of the AR mRNA levels in the testis of the lizard, *Podarcis sicula*. Therefore, we evaluated the expression of TR mRNA by Northern blot analysis and we investigated the role of T_3 on AR mRNA levels in the testis and testicular primary cultures.

2. Materials and methods

2.1. Animals

We utilized adult specimens of male lizards (*Podarcis sicula*) captured in the neighborhood of Naples (Italy) during March (pre-reproductive period), when the natural photoperiod is 11 h daylight with a temperature ranging from 12 to 14°C.

2.2. Chemicals

Glassware was sterilized and RNAse free. Triiodothyronine (T_3) and testosterone (T) were purchased from Sigma (Sigma-Aldrhich, Italy). All other materials were of molecular biology grade. α^{32}-P-dCTP (3000 Ci/mmol) was purchased from Amersham International (Amersham, Italy). Restriction enzymes were from New England Biolabs (Beverly, USA).

2.3. Animal treatments

Lizards were randomly divided into three groups ([a], [b], [c]) ($n = 25$/group). Group [a] was maintained in terraria under environmental photothermal regimes (control group). Groups [b], [c] were kept for 15 days in terraria under experimental phothermal conditions (24°C and 15 h daylight). Furthermore group [b] was treated every two days with intraperitoneal injection (100 μl) of vehicle (saline) while group [c] was treated every two days with intraperitoneal injection (100 μl) of T_3 (10^{-5} M). The experimental phothermal conditions (24°C and 15:9 L:D) utilized in our study induce a complete gonadal recovery many weeks earlier than the usual period [2]. The animals were fed on meal worms and fresh vegetables ad libitum.

At the end of the experiments, the lizards were killed under anesthesia, and the gonads were aseptically excised. Some testes were frozen in liquid nitrogen for RNA extraction, the others were washed in balanced saline solution for preparation of primary cell cultures.

2.4. Preparation of primary cultures of testis cells

Establishment of primary cultures of testis cells was carried out as described in Cardone et al. [6]. The analysis of cell types present in all preparations indicated that at least 80% of the total cells were primary and secondary spermatocytes and spermatids, whereas spermatogonia were about 8% and spermatozoa were absent. Interstitial cells and peritubular cells were about 2% and 10%, respectively, whereas Sertoli cells were rarely seen. The viability of the cells was evaluated by using the expression of cytoskeletal β-actin mRNA as control and was always $>90\%$.

2.5. RNA extraction

Total cellular RNA was extracted following Chomczynski and Sacchi [21] with minor modifications. The yield and quality of RNA were assessed by the 260/280 nm optical density ratio (1.93 ± 0.05) and by electrophoresis under non-denaturating conditions on 1.2% agarose gels.

2.6. Northern blot analysis of TR expression

For Northern analysis, 30 μg of total RNA, isolated from whole lizard testes, was subject to electrophoresis on 1.2% agarose-formaldehyde gel. Single strand (λ–DNA/Hind III digest (23.130–0.564 kb, Stratagene, La Jolla, USA) and RNA molecular-weight markers (7.4–1.6 kb, Boehringer) were used to determine the sizes. Sample and markers were transferred overnight onto nylon membrane (Nytran, Schleicher & Schuell) using 10 × SSC (SSC: 0.015 M trisodium citrate, 0.15 M NaCl, pH 7.0). Filters were baked for 30 min/80°C in a vacuum oven and exposed to UV irradiation (254 nm/2 min). Prehybridization was performed at 42°C for 4 h in 50% deionized formamide, 5 × SSC, 0.1% SDS, 0.05 M phosphate buffer (pH 6.8), 0.005 M EDTA, 5 × Denhardt's solution [Denhardt's solution: 1% (w/v) ficoll, 1% (w/v) polyvinylpyrrolidone, 1% (w/v) BSA], 100 μg/ml yeast tRNA. Hybridization buffer was as above. A 840 bp fragment (Pst 1) from Xenopus TR α form cDNA (xTRα) [22] and a 796 bp fragment (Hinf I) from Xenopus TR ß form cDNA (xTRß) [22] were used as probe. Probes were labeled with α-^{32}P-dCTP by random priming to a specific activity of 5×10^8 cpm/μg [23]. Hybridizations were performed at 42°C overnight. Filters were washed twice with 2 × SSC, 0.1% SDS at 68°C, twice with 0.2 × SSC, 0.1% SDS at 68°C and once with 0.1 × SSC, 0.1% SDS at 72°C. Dried filters were exposed to X-ray film (Fuji HR-H) for 48–96 h.

2.7. Effect of in vivo different experimental conditions on AR mRNA expression in lizard testis

Total cellular RNA, isolated from lizard testis of different experimental groups ([a], [b], [c]) was subjected to analysis of the relative amounts of AR mRNA expression. For this purpose, the analysis of RNA was performed by quantitative slot-blot technique as described elsewhere [24,25]. Thus, total RNA (15 μg/sample) was bound to nylon membrane (Nytran, Schleicher & Schuell) in a slot-blot apparatus (Schleicher & Schuell). Slot-blots were performed in duplicate and hybridized as described by Cardone et al. [6]. Prehybridizations, hybridizations and washes were carried out as above.

The relative amounts of AR mRNA were determined by densitometric scanning of the autoradiograms and normalized to corresponding values of β-actin mRNA.

2.8. Effect of T on AR mRNA expression in primary cultures of testis cells within different experimental groups

Testicular cells from the different experimental groups ([a], [b], [c]) were cultured in the presence or absence (control) of T (10^{-8} M). The incubations were performed for 8, 24, and 48 h, replacing the culture medium and drug every 24 h. At the end of each experimental time, the cells were washed and RNA was extracted as before.

Total RNA (15 μg/sample) was bound to nylon membrane in a slot-blot apparatus. Duplicate filters were hybridized to rAR and β-actin probes, respectively [6]. The experiment was performed three times using three different cell preparations. Prehybridizations, hybridizations and washes were carried out as above.

Two different types of analysis were performed by slot-blot method depending on the way of samples loading. The first one was the analysis of steady-state levels of AR and β-actin mRNAs in T-exposed and unexposed cells of each experimental group ([a], [b], [c]). For this purpose, RNA samples at each experimental time (i.e., in [a] group: T-exposed cells at 0, 8, 24, 48 h) were loaded consecutively and the densitometric values were obtained by scanning the slot-blot hybridization signals. Data were expressed as relative amounts of AR mRNA and β-actin mRNA with respect to total amount of signals (i.e., in [a] group T-exposed cells: $0 + 8 + 24 + 48 = 100\%$). With the second way of analysis we studied the differences of AR mRNA levels between T-exposed and unexposed cells from time to time. For this purpose, samples having the same experimental time (i.e., in [c] group at 24 h: control cells and T-exposed cells) were loaded consecutively. The densitometric values were expressed as relative amounts of AR mRNA with respect to the total amount of signals (i.e., in [c] group at 24 h: control cells + T-exposed cells = 100%).

2.9. Analysis of AR mRNA expression in primary cultures of testis cells between the different experimental groups

We also examined the differences in AR mRNA levels between the T-exposed cells from the three experimental groups ([a] [b] [c]). For this purpose, RNA samples having the same experimental time (i.e., at 24 h: [a], [b], [c]) were loaded consecutively. The densitometric values, obtained by scanning the signals at each time, were expressed as relative amounts of AR mRNA with respect to the total signals (i.e., at 24 h: [a] + [b] + [c] = 100%).

2.10. Statistics

Significance of differences were evaluated by Duncan test for multigroup comparisons. Data were expressed as mean ± SD.

3. Results

3.1. Northern blot analysis of TR expression

Fig. 1 shows the Northern blot detection of TR mRNA in lizard testes from specimens captured during the pre-reproductive period (March). The probe used for TRα mRNA recognizes both TRα1 and TRα2 forms. The molecular sizes of these transcript were 5.0 kb for TRα1 and 2.6 kb for TRα2 mRNA, a value similar to that reported for many mammals. A minor 6.0 kb band was also observed; it reportedly represents unprocessed heteronuclear RNA, encoding unspliced primary transcripts of RNA prior to their processing into the mature TRα1 and TRα2. As far as the occurrence of TRß mRNA in lizard testis is concerned, no TRß transcript was observed (data not shown).

It is worth to note that the probes used in this study contain both the steroid and the DNA binding domains and despite the high stringency conditions, it resulted evident that the testis of the lizard strongly expressed TRα mRNA.

3.2. Effect of different in vivo experimental conditions on AR mRNA expression in lizard testis

Fig. 2 shows the levels of AR mRNA expression in lizard testis between the different experimental groups ([a], [b], [c]). Densitometric analysis of slot-blot hybridization, expressed as relative amounts of AR mRNA, indicated that in the lizard testis, experimental photothermal conditions (24°C and 15 h daylight) were able to stimulate the AR mRNA expression above the control levels. After in vivo T_3-injection, the AR mRNA levels were further enhanced, indicating that T_3 was in some way able to increase AR mRNA expression.

3.3. Effect of T on AR mRNA expression in primary cultures of testis cells from different experimental groups

In vitro experiments confirmed that exposure to androgens affected AR mRNA levels in all experimental groups. Fig. 3 illustrates the steady-state levels of AR and ß-actin mRNAs and the time course analysis of AR mRNA expression in primary cultures of testis cells obtained from animals of control group ([a]). The AR mRNA expression increased in T-exposed cells while there was a continuous decrease in control cells (Fig. 3A). In Fig. 3B, the autoregulatory effect of T is also evident.

The steady-state levels of AR and ß-actin mRNAs and the time course analysis of AR mRNA expression in primary cultures of testis cells obtained from animals maintained under experimental photothermal conditions (24°C and 15 h daylight; [b]) are shown in Fig. 4. Also in this group, the exposure of the cells to T, increase the AR mRNA levels. In fact, the time course analysis (Fig. 4B) show that at the beginning of the experiment (time 0), no significant differences in AR mRNA levels were observed, both in control and T-exposed cells. AR mRNA was significantly increased in T-exposed cells at 8, 24, and 48 h after treatment. A similar trend was observed in primary cultures of testis cells obtained from animals maintained at 24°C and injected with T_3([c]) (Fig. 5). The steady-state levels (Fig. 5A) shows that T increased AR mRNA levels,

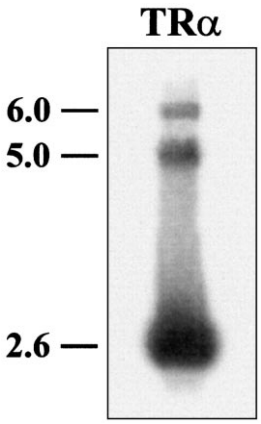

Fig. 1. Northern blot analysis of thyroid hormone receptor mRNA (TR mRNA) in lizard testis. Total RNA (30 μg), obtained from the whole testis, was subjected to electrophoresis under denaturating conditions and transferred to nylon membrane. The blot was hybridized with a random-labeled 840 bp fragmen (Pst 1) from Xenopus TRα form (xTRα) [22] or to 796 bp fragment (Hinf I) from Xenopus TRß form cDNA (xTRß) [22] (data not shown). The sizes of the specific TRα transcripts are indicated on the left side of the figure.

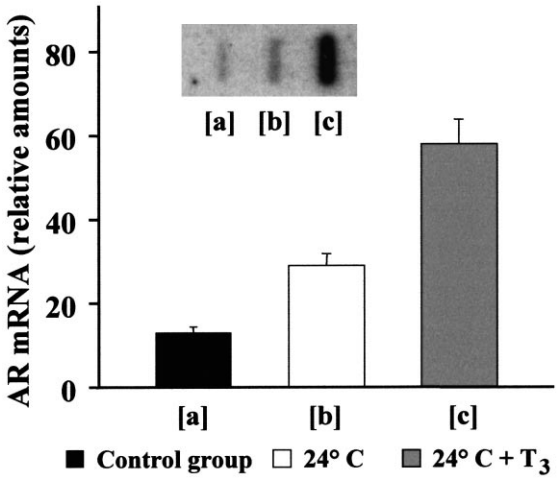

Fig. 2. Levels of AR mRNA expression in lizard testis between the different experimental groups ([a], [b], [c]). Insert: Representative autoradiogram of a slot-blot hybridization of the AR mRNA. The relative concentrations were determined by densitometric analysis of slot-blot hybridization and expressed as relative amounts of AR mRNA with respect to the total signals. Values are the means ± SD.

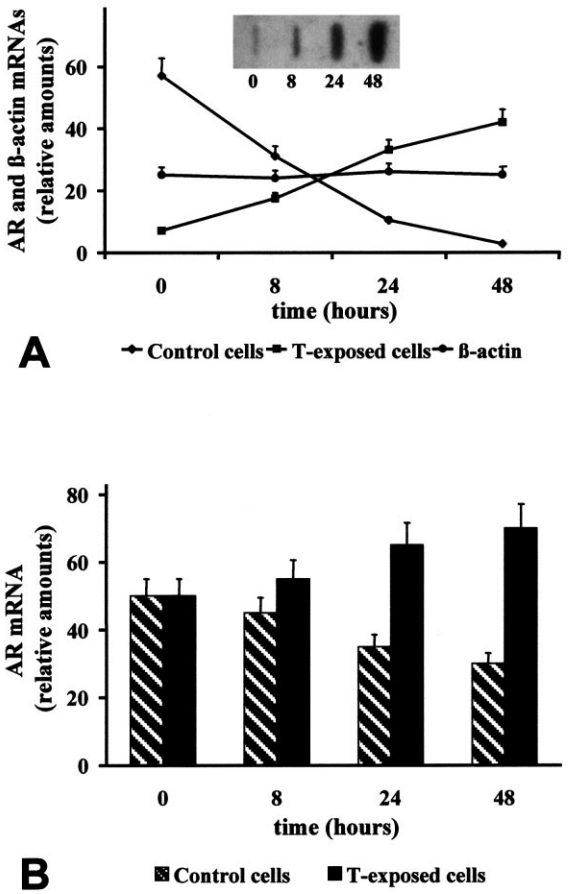

Fig. 3. Analysis of AR and ß-actin mRNAs in primary cultures of testis cells of experimental group [a] (control group). The testicular cells were cultured in the presence or absence (control) of testosterone (T 10^{-8} M). The relative values were determined by slot-blot hybridization. Values are the means ± SD. A: Steady-state levels of AR and ß-actin mRNAs as a function of the time. Values for ß-actin are shown for T-exposed cells. Insert: Typical autoradiogram of a slot-blot of total RNA isolated from T-exposed cells, probed for AR. B: Time-point analysis of AR mRNA between the control and T-exposed cells. The values were obtained by scanning the hybridization signals at each time and were expressed as relative amounts with respect to the total signals.

while there was a continuous decrease in control cells. These results are also confirmed by time point analysis (Fig. 5B), although such a decrease was less evident than that seen in control cells from animals maintained at 24°C without T_3 treatment (Fig. 4B). In all the experiments the levels of ß-actin mRNA did not show variation between the control and T-exposed cells.

3.4. Analysis of AR mRNA expression in primary cultures of testis cells between the different experimental groups

The stimulatory effect of in vivo T_3-priming on AR mRNA levels was evident from the results of time point analysis of T-exposed testis cells from different experimental groups ([a], [b], [c]) (Fig. 6). Densitometric analysis of slot-blot hybridization, expressed as the relative amount of AR mRNA, indicated that the highest values of AR mRNA were found in the T_3-primed group. In fact, in T-exposed testis cells from animals injected in vivo with T_3, AR mRNA expression was higher than the other two groups at any time.

4. Discussion

It has been clearly demonstrated that hormones do not act separately, but they operate jointly in complex signalling networks [20,26–29]. An example of hormonal interplay is represented by the participation of

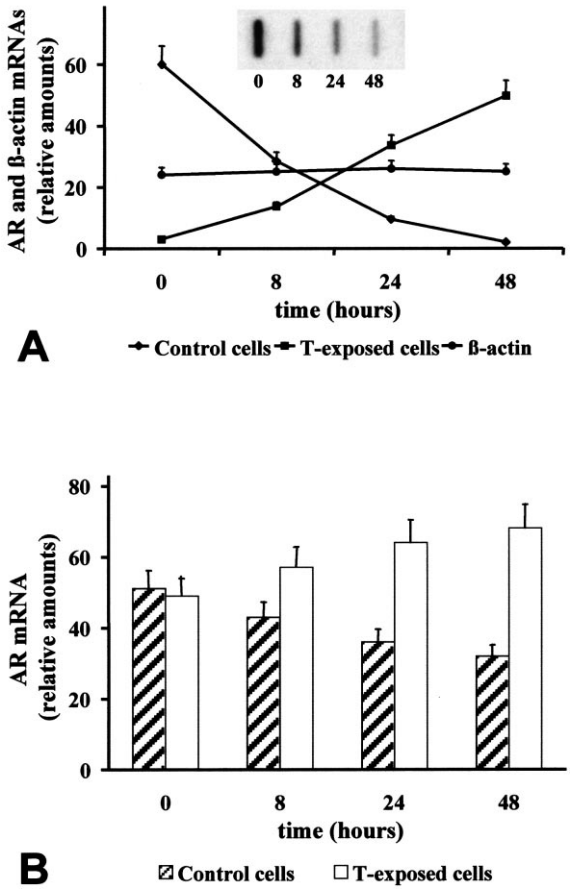

Fig. 4. Analysis of AR and ß-actin mRNAs in primary cultures of testis cells of experimental group [b] (24°C and 15 h daylight). The testicular cells were cultured in the presence or absence (control) of testosterone (T 10^{-8} M). The relative values were determined by slot-blot hybridization. Values are the means ± SD. A: Steady-state levels of AR and ß-actin mRNAs as a function of the time. Values for ß-actin are shown for T-exposed cells. Insert: Typical autoradiogram of a slot-blot of total RNA isolated from control cells, probed for AR. B: Time-point analysis of AR mRNA between the control and T-exposed cells. The values were obtained by scanning the hybridization signals at each time and were expressed as relative amounts with respect to the total signals.

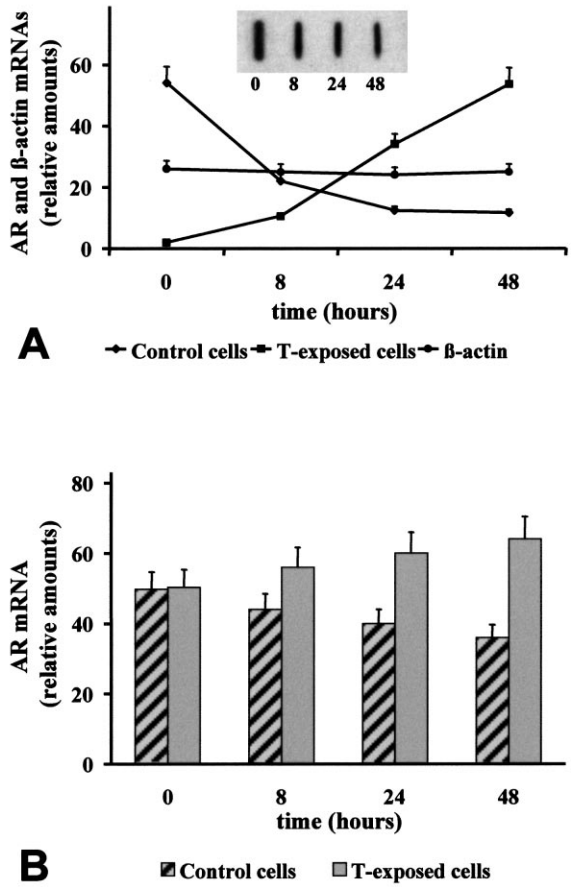

Fig. 5. Analysis of AR and ß-actin mRNAs in primary cultures of testis cells of experimental group [c] (24°C and 15 h daylight plus T_3 injection). The testicular cells were cultured in the presence or absence (control) of testosterone (T 10^{-8} M). The relative values were determined by slot-blot hybridization. Values are the means ± SD. A: Steady-state levels of AR and ß-actin mRNAs as a function of the time. Values for ß-actin are shown for control cells. Insert: Typical autoradiogram of a slot-blot of total RNA isolated from control cells, probed for AR. B: Time-point analysis of AR mRNA between the control and T-exposed cells. The values were obtained by scanning the hybridization signals at each time and were expressed as relative amounts with respect to the total signals.

thyroid hormones in multi-hormonal regulation of many physiological processes [19,20,26,30–33]. Recently, it has been established that T_3 can increase Sertoli cell protein synthesis, providing further evidence that thyroid hormone has a fundamental role in the regulation of growth and differentiation of mammalian testis [34]. Binding studies and in situ hybridization indicate that Sertoli cells express TR and its mRNA during the neonatal period [35,36]. TR is encoded by two different genes, α and ß; the alternative splicing of gene primary transcripts leads to the production of at least four distinct mRNA species generating separate TR isoforms (TRα1, TRα2, TRß1, TRß2). The TRα1 and TRß isoforms bind T_3 and appear to operate as transcriptional activators, while the physiological significance of TRα2, a non-T_3-binding variant, remains unknown [37,38].

The lizard testis expresses both TRα1 and TRα2 mRNA, having molecular sizes of 5.0 and 2.6 kb, respectively, similarly to what has been found in prepuberal rats [36,39]. The other major functional TR mRNA (TRß1), detected in rat Sertoli cells by PCR [36,40], has never been observed. However, the hybridization conditions and the molecular weight of the transcripts are consistent with the presence of TRα1 and TRα2 mRNAs in the testis of sexually mature lizards.

In mammals, adult testes are devoid of both T_3 nuclear binding activity [35,41] and TRα1 and TRß1 mRNA isoforms, while they exclusively express the non-hormone binding isoforms TRα2 [36,42,43]. On the other hand, in rat testes, TRα1 mRNA expression [36] and TRß1 transcript [40] have been detected only in immature Sertoli cells. As indicated by maximal binding capacity, the concentration of TR in whole testes is highest during the fetal and perinatal period and decreases significantly until puberty, virtually disappearing in the adult [35]. The presence of TR in fetal, perinatal and prepuberal rat testes suggests that T_3 might regulate the growth of the seminiferous epithelium by modulating some different functions of Sertoli cells [44]. Although, our results are in contrast with those report in mammals, the occurrence of TRα mRNA in adult lizard testes indicates that the thyroid hormone would have a direct effect on these cells. It has been proposed that the evolution of the thyroid function might have been paralleled by the endocrine control of reproduction and that the basic function for thyroid hormones might be associated primitively with

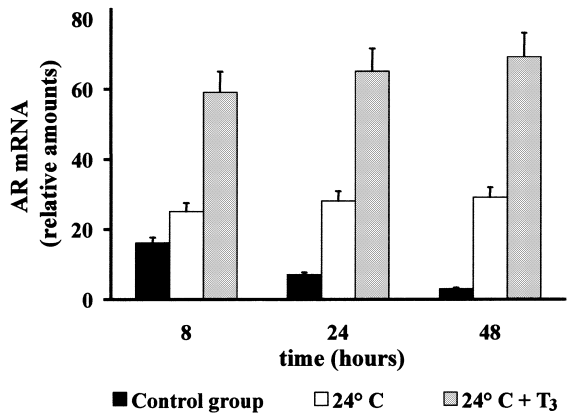

Fig. 6. Time-point analysis of AR mRNA in primary cultures of testis cells in all experimental group ([a], [b], [c]). The testicular cells were cultured in the presence of testosterone (T 10^{-8} M). The relative values were determined by slot-blot hybridization. The values were obtained by scanning the hybridization signals between the different experimental group at each time and were expressed as relative amounts with respect to the total signals. Values are the means ± SD.

gonadal maturation. Generally, the thyroid function in reptiles varies in proportion to changes in temperature, although the cause-effect relationships are still obscure [45]. Seasonal changes in thyroid function have been correlated with a number of reproductive events (spermatogenesis, ovulation, mating) in lizards, snakes, and turtles [46]. In the lizard, thyroidectomy causes gonadal regression, which can be overcome by thyroid hormone administration [47]. Moreover, hyperthyroidism, induced by thyroid hormone injection into intact lizards, blocks testis growth [15]. The effect of thyroid hormone, however, shows seasonal variations, which are greater when testes are fully active than during recrudescence [16]. The marked seasonality of thyroidal activities in reptiles further supports an involvement of this gland in the reproductive cycle [46]. Apart from these considerations, the expression of TRα mRNA in lizard testis also provides evidence that thyroid hormones can directly modulate AR mRNA expression in testicular cells. Recent works have indicated that T_3-treatment regulates AR and ER content in cultured Sertoli cells from peripuberal rats [48] and results in increased AR mRNA levels in the cultures of the same cells from 5-day-old rats [39]. In this respect, our in vivo experiments showed that, in lizards, either temperature alone (24°C) or temperature and T_3 significantly stimulated AR mRNA levels above the control values. However, temperature and T_3 affected AR mRNA expression levels more markedly than the temperature alone, indicating that T_3 is able to up-regulate AR mRNA expression. It is clear that the increase in steady-state AR mRNA levels in lizard testes is due to a specific direct effect of T_3 on AR mRNA levels, rather than to a general stimulatory effect of T_3 on testis maturation. In fact, the analysis of germ cell percentage conducted on histological sections of both experimental groups did not reveal great differences (data not shown).

In vitro experiments confirmed previous results showing that exposure to androgens enhances AR mRNA levels [6]. It is worth noting that our previous results were gained using testicular cells from animals in full breeding season, while the present study was carried out during the pre-reproductive period (March). The results show the same pattern although the absolute values could be different, indicating that in this period the testis is able to respond to androgens up-regulating the AR mRNA. In fact, in T-exposed cells, AR mRNA significantly increased after 8, 24, and 48 h, with respect to the unexposed (control) cells in all experimental groups. The differences of AR mRNA levels, between unexposed and T-exposed cells from animals maintained at 24°C and T_3-primed, however, were less evident with respect to the other two experimental groups. This result was probably due to the additional effect of the in vivo T_3-priming and a greater thyroid activity in the specimens of this experimental group. It is known that, in lizards from temperate regions, thyroid activity is highest during the warmer seasons. Artificial lowering of the environmental temperature during summer causes a reduction in thyroid activity. Lizards maintained in the laboratory at high temperatures (35°C) exhibit greater thyroid function than when maintained at 15°C [44]. The stimulatory effect of T_3-priming on AR mRNA levels is evident from our results of time point analysis of T-exposed cells, obtained from the different experimental groups. These results indicate that the highest values of AR mRNA were found in the T_3-primed group. In fact, time-course analysis showed that, in T-treated cells from animals exposed in vivo to T_3, AR mRNA levels increased after 8, 24, and 48 h, although, the steady-state levels showed a rapid accumulation of AR mRNA in each group.

The effects of T_3 and T suggest that these hormones act in different pathways. Since receptors play a cental role in the action of hormones, T_3, binding its own receptor (TR), is able to stimulate AR mRNA levels and in turn influence the androgen responsiveness of testis cells during spermatogenesis. It is conceivable that thyroid hormone might act with other factors in the regulation of seasonal spermatogenetic cycle. In this respect it is worth to note that in a previous work carried out in our laboratory, we found that estrogens strongly down-regulated the expression of AR mRNA in primary cultures of lizard testis cells [6]. Increasing evidence in support of cross-talk between nuclear receptors and various types of signals has been observed [19,26–29,49,50]. Therefore, it appears that the cooperation of a number of hormonal (T, E, T_3) and, probably, non-hormonal signals, is required for regulation of spermatogenetic cycle in the lizard. Taken together, our studies indicate the importance of interplay between different ligands of the steroid/thyroid hormone nuclear receptor gene family acting by modulating the expression of the gene encoding one of the members of this superfamily.

Acknowledgements

We wish to thank Mr. Giuseppe Falcone for typing and setting all the illustrations of the paper. This work was supported by MURST, Progetto Nazionale (Prof. R. Geremia).

References

[1] F. Angelini, G. Ghiara, Reproductive models and strategies in vertebrate evolution, It J. Zool. 51 (1984) 121–203.
[2] F. Angelini, V. Botte, Spermatogenesis in reptiles, dynamic and

regulatory aspect, in: R. Dallai (Ed.), Sex Origin and Evolution, vol 6, Mucchi Selected Symposia and Monographs UZI, Modena, 1992, pp. 211–230.
[3] F. Angelini, O. Picariello, V. Botte, Influence of photoperiod and temperature on the testicular activity of the lizard *Lacerta s. sicula* Raf, It. J. Zool. 43 (1976) 111–123.
[4] S. Andò, M.L. Panno, G. Ciarcia, E. Imbrogno, M. Buffone, E. Beraldi, D. Scisci, F. Angelini, V. Botte, Plasma sex hormone concentration during the reproductive cycle in the male lizard *Podarcis s. sicula*, J. Reprod. Fert. 90 (1990) 353–360.
[5] S. Andò, G. Ciarcia, M.L. Panno, E. Imbrogno, C. Tarantino, M. Buffone, E. Beraldi, F. Angelini, V. Botte, Sex steroid levels in the plasma and testis during the reproductive cycle of the lizard *Podarcis s. sicula* Raf, Gen. Comp. Endocrinol. 85 (1992) 1–7.
[6] A. Cardone, F. Angelini, B. Varriale, Autoregulation of estrogen and androgen receptor mRNAs and down regulation of androgen receptor mRNA by estrogen in primary cultures of lizard testis cells, Gen. Comp. Endocrinol. 110 (1998) 227–236.
[7] A.R. Chowdhury, U. Arora, Role of thyroid in testicular development of immature rat, Arch. Andr. 12 (1984) 49–51.
[8] S. Francavilla, G. Cordeschi, G. Properzi, L. Di Cicco, E.A. Jannini, S. Palmero, E. Fugassa, B. Loras, M. D'Armiento, Effect of thyroid hormone on the pre- and post-natal development of the rat testis, J. Endocrinol. 129 (1991) 35–42.
[9] C. Longcope, The male and female reproductive systems in hypothyroidism, in: S.H. Ingbar, L.E. Braverman (Eds.), The Thyroid, 6th ed., Lippinincot, Philadelphia, 1991, pp. 1152–1155.
[10] P.S. Cooke, E. Meisami, Early hypothyroidism in rats causes increased adult testis and reproductive organ size but does not change testosterone levels, Endocrinology 129 (1991) 237–243.
[11] P.S. Cooke, R.A. Hess, J. Porcelli, E. Meisami, Increased sperm production in adult rats after transient neonatal hypothyroidisms, Endocrinology 129 (1991) 244–248.
[12] L.H. van Haaster, F.H. de Jong, R. Docter, D.G. de Rooij, The effect of hypothyroidism on Sertoli cell proliferation and differentiation and hormone levels during testicular development in the rat, Endocrinology 131 (1992) 1574–1576.
[13] J.D. Kirby, A.E. Jetton, P.S. Cooke, R.A. Hess, D. Bunick, J.F. Ackland, F.W. Turek, N.B. Schwartz, Developmental hormonal profiles accompanying the neonatal hypothyroidisms-induced increase in adult rat testis size and sperm production in the rat, Endocrinology 131 (1992) 559–565.
[14] M. Jallageas, B.K. Follet, I. Assenmacher, Thyroid-gonadal interactions during the postnuptial phase of the sexual cycle in male ducks, Gen. Comp. Endocrinol. 34 (1978) 68–69.
[15] M.M. Plowman, W.G. Lynn, The role of the thyroid in testicular function in the gecko, *Cleonyx variegatus*, Gen. Comp. Endocrinol. 20 (1973) 342–346.
[16] C.M. Haldar-Misra, J.P. Thapliyal, Thyroid in reproduction in reptiles, Gen. Comp. Endocrinol. 43 (1981) 537–542.
[17] M.R. Warren, Studies on the effect of experimental hyperthyroidism on the adult frog *Rana pipiens*, J. Exp. Zool. 83 (1940) 127–159.
[18] S.A. Sakr, Effect of thyrosine on testicular activity in the toad *Bufo regularis*, Folia Morphol. (Warsz) 36 (1988) 256–259.
[19] S. Ulisse, J.R. Tata, Thyroid hormone and glucocorticoid independently regulate the expression of estrogen receptor in male Xenopus liver cells, Mol. Cell. Endocrinol. 105 (1994) 45–53.
[20] B. Varriale, T. Esposito, E. Astore, A. Cardone, Estrogen, glucocorticoid and thyroid hormone receptor mRNAs: relationships with androgen receptor gene expression in primary cultures of male hamster Harderian gland cells, in: E.W. Roubos, S.E. Wendelaar Bonga, H. Vaudry, A. De Loof (Eds.), Recent Developments in Comparative Endocrinology and Neurobiology, Shaker, Maastricht, The Netherlands, 1999, pp. 40–43 in press.
[21] P. Chomczynski, N. Sacchi, Single step method of RNA isolation by guanidinium thiocyanate phenol chloroform extraction, Anal. Biochem. 162 (1987) 156–159.
[22] Y. Yaoita, Y.B. Shi, D.D. Brown, *Xenopus laevis* α and β thyroid hormone receptors, Proc. Natl. Acad. Sci. USA 87 (1990) 7090–7094.
[23] A.P. Feinberg, B. Vogelstein, A technique for radiolabeling DNA restriction fragments to high specific activity, Anal. Biochem. 137 (1984) 266–267.
[24] B. Varriale, J.R. Tata, Autoinduction of estrogen receptor is associated with FOSP-1 mRNA induction by estrogen in primary cultures of Xenopus oviduct cells, Mol. Cell. Endocrinol. 71 (1990) R25–R31.
[25] B. Varriale, I. Serino, The androgen receptor mRNA is upregulated by testosterone in both Harderian gland and thumb pad of the frog, *Rana esculenta*, J. Steroid Biochem. Mol. Biol. 51 (1994) 259–265.
[26] E.E. Baulieu, P. Kelly, Hormones. From Molecules to Disease, Hermann, Paris, 1990.
[27] H. Cho, B.S. Katzenellenbogen, Synergistic activation of estrogen receptor-mediated transcription by estradiol and protein kinase activators, Mol. Endocrinol. 7 (1993) 441–452.
[28] E. Imai, J.N. Miner, J.A. Mitchell, K.R. Yamamoto, D.K. Granner, Glucocorticoid receptor-cAMP response element-binding protein interaction and the response of the phosphoenolpyruvate carboxykinase gene to glucocorticoids, J. Biol. Chem. 268 (1993) 5353–5356.
[29] B.W. O'Malley, M.J. Tsai, in: M.G. Parker (Ed.), Steroid Hormone Action, IRL Press, Oxford, 1993, pp. 45–63.
[30] R. Pitt-Rivers, J.R. Tata, The Thyroid Hormones, Pergamon Press, London, 1959.
[31] A. Gorbman, H.A. Bern, A Textbook of Comparative Endocrinology, Wiley, New York, 1962.
[32] J.H. Oppenheimer, H.H. Samuels, Molecular Basis of Thyroid Hormone Action, Academic Press, New York, 1983.
[33] J.R. Tata, Gene expression during metamorphosis: an ideal model for post-embryonic development, Bioessays 15 (1993) 239–248.
[34] S. Palmero, G. Bardi, F. Bolla, E. Fugassa, Influence of thyroid hormone on Sertoli cell protein metabolism in the prepubertal pig, Boll. Soc. It. Biol. Sper. 72 (1996) 163–170.
[35] E.A. Jannini, M. Olivieri, S. Francavilla, A. Gulino, E. Ziparo, M. D'Armiento, Ontogenesis of nuclear 3,5,3′-triiodothyronine receptor in the rat testis, Endocrinology 126 (1990) 2521–2526.
[36] E.A. Jannini, S. Dolci, S. Ulisse, V.M. Nikodem, Developmental regulation of the thyroid hormone receptor α1 mRNA expression in rat testis, Mol. Endocrinol. 8 (1994) 89–96.
[37] J.H. Oppenheimer, Thyroid hormone action at the molecular level, in: L.E. Braverman, R.D. Utiger (Eds.), The Thyroid, 6th ed., Werner and Ingbar's, Lippinincot, Philadelphia, 1991, pp. 204–224.
[38] M.A. Lazar, Thyroid hormone receptors: multiple forms, multiple possibilities, Endocr. Rev. 14 (1993) 184–193.
[39] N.K. Arambepola, D. Bunick, P.S. Cooke, Thyroid hormone effect on androgen receptor messenger RNA expression in rat Sertoli and peritubular cells, J. Endocrinol. 156 (1998) 43–50.
[40] S. Palmero, P. De Marco, E. Fugassa, Thyroid hormone receptor ß mRNA expression in Sertoli cells isolated from prepubertal testis, J. Mol. Endocrinol. 14 (1995) 131–134.
[41] J.H. Oppenheimer, H.L. Schwartz, M.I. Surks, Tissue differences in the concentration of triiodothyronine nuclear binding sites in the rat: liver, kidney, pituitary, heart, brain, spleen and testis, Endocrinology 95 (1974) 897–903.
[42] K.A. Strait, H.L. Schwartz, A. Perez-Castillo, J.H.

Oppenheimer, Relationship of c-erbA mRNA content to tissue triiodothyronine nuclear binding capacity and function in developing and adult rats, J. Biol. Chem. 265 (1990) 10514–10521.

[43] C.P. Barsano, Z. Iqbal, G.L. Pullen, E. Munoz, S.P. Singh, Tissue- specific differences in the compartmentalization of rat nuclear triiodothyronine receptors, Acta Endocrinol. 122 (1990) 181–190.

[44] E.A. Jannini, S. Ulisse, M. D'Armiento, Thyroid hormone and male gonadal function, Endocr. Rev. 16 (1995) 443–459.

[45] D.O. Norris, Vertebrate Endocrinology, 3rd ed., Academic Press, New York, 1997, pp. 1–393.

[46] W.G. Lynn, The thyroid, in: C. Gans, T.S. Parson (Eds.), Biology of Reptilia. Morphology C, vol. 3, Academic Press, New York, 1970, pp. 201–234.

[47] A. Kar, A. Chandola, Seasonality in birds and reptiles: the involvment of thyroxine and triiodothyronine, in: B.K. Follet, S. Ishii, A. Chandola (Eds.), The Endocrine System and the Environment, Japanese Science Society Press/Springer-Verlag, Berlin, 1984, pp. 117–126.

[48] M.L. Panno, D. Sisci, M. Salerno, M. Lanzino, V. Pezzi, E.G. Morrone, L. Mauro, S. Palmero, E. Fugassa, S. Andò, Thyroid hormone modulates androgen and oestrogen receptor content in the Sertoli cell of the peripubertal rat, J. Endocrinol. 148 (1996) 43–50.

[49] R. Schule, M. Muller, C. Kaltschmidt, R. Renkawitz, Many trancription factors interact synergistically with steroid receptors, Science 242 (1988) 1418–1420.

[50] B.S. Baker, J.R. Tata, Prolactin prevents the autoinduction of thyroid hormone receptor mRNAs during amphibian metamorphosis, Dev. Biol. 146 (1992) 463–467.