international journal of andrology, 28:99–106 (2005) doi: 10.1111/j.1365-2605.2005.00516.x

Ontogenetic profile and thyroid hormone regulation of type-1 and type-8 glucose transporters in rat Sertoli cells

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Summary

The glucose transporters (GLUTs) gene encode glycoproteins responsible for facilitating transfer of glucose across plasma membrane. In testis, different members of this family are present. In particular the main GLUT mRNA expression within the adult testis is the type 8, while type 1 is more expressed in prepubertal testis. Thyroid hormone, which receptors and function have been characterized in the testis, plays a crucial role in the cellular energetic metabolism. In fact, in the immature Sertoli cells, GLUT1 is up regulated by L-triiodothyronine (T_3). The aim of this paper is to investigate the expression profile of GLUT1 and GLUT8 in the testis during development and in adulthood and analyse the role of T_3 on their expression. To analyse the expression of GLUT8 and GLUT1 we performed Northern blot and RT-PCR experiments in the whole testis and in Sertoli cells from rats of different ages. Treatments in vivo and in vitro with T_3 were used to study the effect of thyroid hormones on GLUT1 and GLUT8 expression. The activity of the rat GLUT1 promoter and its regulation by T_3 was studied with transient transfections in gonadal and non-gonadal cell lines and in primary Sertoli cell cultures. GLUT8 is expressed at a low level in the prepubertal testis and Sertoli cells and does not appear to be under T_3 control. GLUT1 is the predominant form in immature Sertoli cells. The effect of T_3 on its mRNA accumulation was quantified and confirmed by RT-PCR (control: 0.65 ± 0.17; T_3: 1.23 ± 0.04, arbitrary units, $p < 0.05$). However, transfection experiments showed that T_3 does not directly regulate GLUT1 promoter in any cell line tested. This is confirmed by the evidence that, upon extensive analysis, the rat GLUT1 promoter and the first intron sequence do not shows any thyroid responsive elements. Our data demonstrate that GLUT1 and GLUT8 are both expressed in prepubertal testis, but only GLUT1 is regulated by T_3. In addition, we found that the effect of T_3 cannot be attributed to its action on GLUT1 promoter.

Keywords: glucose, glucose transporter, Sertoli cell, thyroid hormone

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Introduction

Growing evidence both in animals and humans support the role of thyroid hormones and thyroid hormone nuclear receptors (TRs) in the development of male gonadal tissues. As germ cell maturation requires lactate (Jutte *et al.*, 1982)

derived from glucose oxidation in the nursing Sertoli cell (Grootegoed *et al.*, 1986), regulation of the energetic metabolism in the seminiferous microenvironment is crucial. Glucose is hydrophilic and thus cannot penetrate the lipid bilayer. Specific carrier proteins are therefore required to facilitate its diffusion along a concentration gradient. Glucose transporter proteins ($GLUT_s$) are a super-family of transport facilitators with 13 family members, distributed in a wide variety of species (Joost & Thorens, 2001), which help the diffusion of hexoses into mammalian cells (Bell *et al.*, 1990). Different members of this protein family are expressed in the testis: GLUT1 (Ulisse *et al.*, 1992), GLUT3 (Burant & Davidson, 1994), GLUT5 (Burant *et al.*, 1992) and GLUT8 (Doege *et al.*, 2000; Ibberson *et al.*, 2000). In particular, GLUT1 is expressed in both adult and immature rat Sertoli cells, while GLUT8 is the predominant form in the adult testis (Doege *et al.*, 2000; Ibberson *et al.*, 2000) and is prevalently located in the head of spermatozoa (Schurmann *et al.*, 2002).

The prepubertal testis is a target organ for the thyroid hormone (Jannini *et al.*, 1995). In fact, immature Sertoli cell (but not Leydig and peritubular cell) nuclei bind thyroid hormone with high affinity and specificity (Jannini *et al.*, 1990). This binding is the result of the expression of the isoform α_1 of the nuclear thyroid hormone receptor, being the isoform β virtually absent (Jannini *et al.*, 1994). A critical window of TR expression and T_3 action, localized in the foetal, neonatal and prepubertal ages, has been demonstrated (Jannini *et al.*, 1995). Interestingly, a similar expression pattern has been recently demonstrated in the human testis (Jannini *et al.*, 2000).

In Sertoli cells, follicle-stimulating hormone (FSH) is the main energy metabolism regulator (Hall & Mita, 1984), but GLUT1 mRNA expression (Ulisse *et al.*, 1992) and lactate production (Palmero *et al.*, 1989) were stimulated by T_3 in a process requiring *de novo* protein synthesis, suggesting an effect at transcriptional level.

Thus, in Sertoli cells, FSH and thyroid hormone regulate glucose uptake at different levels, through both a fast membrane signalling mechanism and a delayed action through the nuclear level, so that energy requirements of the developing germ cells can be met (Jannini *et al.*, 1995). In fact, neonatal testis fragments cultured in vitro with T_3 show a significant increase in the size of seminiferous cords and in the number of gonocytes, concomitant with a decreased percentage of degenerating germ cells (Jannini *et al.*, 1993).

While the regulation of GLUT8 by thyroid hormone has never been studied, it is interesting to note the cell-specificity of the T_3 effect on the expression of GLUT1. Thyroid hormone increases GLUT1 protein in adipose cells (Matthaei *et al.*, 1995; Romero *et al.*, 2000), but not in other tissues. In fact, a lack of increase of GLUT1 in skeletal muscle and brain (Weinstein *et al.*, 1991), its decrease in cardiac muscle from hyperthyroid rats (Weinstein & Haber, 1992) and its increase in hypothyroid animals (Castello *et al.*, 1994) have been demonstrated. The reasons for the discrepancy between the observations on the role of thyroid hormone on GLUT1 expression obtained from different tissues remain unexplained. In fact, the action mechanism of GLUT1 regulation by T_3 is largely unknown.

In the present study, the expression profiles of two glucose carriers, GLUT1 and GLUT8, were investigated in the testis during development and in adulthood, and the effect of T_3 on their expression was analysed. In addition, the activity of rat GLUT1 promoter was studied in transfection experiments using the monkey COS7 kidney cell line, and in Sertoli cell primary and immortalized cultures, before and after stimulation with T_3.

Materials and methods

Cell cultures

Primary cultures enriched in Sertoli cells were prepared from 8 to 20-day-old Wistar rats as previously described (Galdieri *et al.*, 1981). The cells were plated in MEM containing 0.1% bovine serum albumin (Sigma-Aldrich, St Louis, MI, USA) and incubated in 5% CO_2 at 32 °C. After 48 h culture, the Sertoli cell monolayer was treated with a hypotonic solution (20 mM Tris–HCl, pH 7.4) for 3 min to remove germ cells contamination. In preliminary experiments, the purity of Sertoli cell preparations was assessed after 2 days of culture by staining for peritubular cells with alkaline phosphatase (Palombi & Di Carlo, 1988). In these preparations, Sertoli cells accounted for approximately 90% of the cell population, as judged by phase-contrast examination, with the main contaminant being germ and peritubular cells. The monkey COS7 kidney cell line (Gluzman, 1981) was grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% foetal bovine serum (FBS; Sigma-Aldrich). The adult mouse Sertoli cell lines TM4 (Mather, 1980) were grown in DMEM supplemented with 2.5% FBS and 5% horse serum (Sigma-Aldrich). Preliminary experiments performed by RNase protection assay (Jannini *et al.*, 1994) and RT-PCR demonstrated that this cellular line does not express any TRs (not shown).

Animal treatment

To induce hyperthyroidism, prepubertal and adult male Wistar rats received by intraperitoneal injection 1 μg/g BW/day of T_3 (dissolved in 0.04 N NaOH) for 3 days (Jannini *et al.*, 1992). The control animals received the appropriate dilution of the stock solution of 0.04 N NaOH without thyroid hormone. Hypothyroidism was provoked in adult rats by surgical thyroidectomy followed by feeding with a low iodine test diet and 1% calcium lactate for 1 month (Jannini *et al.*, 1992). The animals were then killed at the indicated times and their testes removed for RNA extraction.

Extraction and analysis of RNA

The Trizol (Invitrogen, Carlsbad, CA, USA) method was used to prepare total RNA from Sertoli cells and TM4 cells. The purity and integrity of RNA preparations were checked spectroscopically and by gel electrophoresis before carrying out the analytical procedure. Total mRNA extracted from primary cultures of Sertoli cells was used in a semiquantitative RT-PCR analysis. First strand complementary DNA was made using, for each sample, 2 μg of total RNA in the presence of M-MuLV reverse transcriptase (Invitrogen) and Poli $d(T)_{12-18}$ primer (Invitrogen). The obtained cDNA was used as a template for the PCR amplification of GLUT1 (655 bp) and rat GAPDH (983 bp) using the following primers: rGLUT1up: 5′-CAA ACA TGG AAC CAC CGC TAT GGA-3′; rGLUT1do: 5′-GGA ACA GCT CCA AGA TGG TGA CC-3′; ratGAPDHup: 5′-TGA AGG TCG GTG TGA ACG GAT TTG GC-3′; ratGAPDHdo: 5′-CAT GTA GGC CAT GAG GTC CAC CAC-3′.

GAPDH was used as an internal control. The PCR was performed with REDTaq DNA polymerase (Sigma-Aldrich; 2.5 units for each reaction), 10 μM of both upstream and downstream primers, and 1.5 mM magnesium chloride. Amplification conditions were 94 °C for 1 min, 55 °C for 1 min and 72 °C for 1 min for 35 cycles in the DNA thermal cycler (PCR Cetus; Perkin-Elmer Corp., Rome, Italy; −20 cycles for GAPDH amplification). The genomic contamination was excluded by running the PCR without RT. Furthermore, the primers were chosen in different exons.

Northern blot

The RNA species were separated by electrophoresis on a 1% agarose, 3% formaldehyde gel and then blotted onto nylon filters (Hybond-N; Amersham, Uppsala, Sweden). The blots were hybridized with a 419-bp fragment of GLUT8 cDNA obtained by RT-PCR (oligos used: MGL-U 5′-TCC TCA CTC AAC ACC AGT ACC AGG AG-3′; MGL-D 5′-TGG GGA GGC TCT GGG TCA GTT TGA AG-3′). The hybridization was carried out at 65 °C for 1 h in QuikHyb solution (Stratagene, La Jolla, CA, USA). The blots were washed once with 2 × SSC and 1% SDS at room temperature for 15 min, once with 1 × SSC and 1% SDS at room temperature for 15 min, and twice with 0.5 × SSC and 1% SDS at 65 °C for 30 min. The hybridized blots were exposed to X-ray film at −80 °C with two intensifying screens. Where indicated the filters were hybridized under the same conditions with a probe against GLUT1 gene. The GLUT1 probe (655 bp) was obtained by RT-PCR using previously mentioned oligos: rGLUT1up and rGLUT1do. Finally, the filters were normalized using a probe against β-actin gene, obtained by RT-PCR (oligos used: β-actin1: 5′-GAC GAC ATG GAG AAG ATC TGG-3′; β-actin2: 5′-GAG GAT GCG GCA GTG GCC AT-3′).

COS7 and TM4 cell lines transfections

One day before the transfection, cells were plated out in DMEM containing serum that was stripped of T_3 by ion-exchange (Samuels *et al.*, 1979). Cells were transfected by calcium phosphate co-precipitation with 3 μg of reporter plasmid pGL2-GLUT1, 100 ng of pRL-SV40 containing *Renilla* (Sea pansy) luciferase (Promega, Madison, WI, USA) as an internal control, and 1 μg of pGS5cTHR$_\alpha$ (TR$_{\alpha1}$) (Carosa *et al.*, 1998). Three μg of 3PAL-Luc, a vector containing a specific T_3 responder element (TRE) (Mangelsdorf & Evans, 1995) was used as a positive control.

The vector pGL2-GLUT1, which contains the 5′-flanking region of the rat GLUT1 gene spanning from −2104 to +138 of the transcription start site cloned in front of luciferase gene in the vector pGL2-basic (Promega), was kindly provided by Dr Ismail-Beigi (Case Western Reserve University, Cleveland, OH, USA) (Behrooz & Ismail-Beigi, 1997).

The total pGS5 vector quantity, with or without the receptor insert, was kept constant in all experiments. The precipitate DNA mixture was left on the cells for 16 h. T_3 (Sigma-Aldrich) was added at the indicate concentrations, and vehicle (ethanol 100% in the first dilution) added to controls. After 48 h culture the cells were lysed with LucLite Substrate Buffer Solution (Packard, Groningen, The Netherlands).

Prepubertal Sertoli cell transfections

Sertoli cells from the primary cultures were transfected in 35 mm plastic tissue culture dishes with the Fugene6 method (Boehringer, Mannheim, Germany) using 1.8 μg of reporter plasmid pGL2-GLUT1, 100 ng of pRL-SV40 containing *Renilla* (Sea pansy) luciferase (Promega) as an internal control, and 1 μg of pGS5cTHR$_\alpha$ (TR$_{\alpha1}$) (Carosa *et al.*, 1998). The total pGS5 vector quantity, with or without the receptor insert, was kept constant in all experiments. T_3 (Sigma-Aldrich) was added at the concentration 10^{-7} M, and vehicle (ethanol 100% in the first dilution) added to controls. After 48 h culture the cells were lysed with LucLite Substrate Buffer Solution (Packard).

Luciferase activity measurement

Firefly luciferase (LA_A) and *Renilla* luciferase (LA_R) activities were measured sequentially using a Firelite Kit (Packard) and a model Lumi Count (Packard). The relative luciferase activity (RLA) was calculated as: $RLA = LA_A/LA_R$. Transfections were performed in duplicate and the average values from at least three experiments are shown.

Statistical analysis

Results are expressed as the mean ± SE of at least three experiments and values were statistically compared using the Student's *t*-test. Results were determined to be significantly different if p values were <0.05.

Results

Ontogenetic profile and in vivo thyroid hormone regulation of GLUTs

Type 1 and type 8 GLUT are both expressed in the testis, but with different ontogenetic control (Fig. 1). Type 8 is the predominant form found in the adult testis (Ibberson *et al.*, 2000; Scheepers *et al.*, 2001). In testes from 1-day-old animals, a low level of GLUT8 was detectable only after 10 days of film exposure (Fig. 1B). Its expression remained low during development and showed a large increase in adulthood.

In contrast, GLUT1 was expressed at a low level in the 1-day-old testis, but increased rapidly, reaching almost adult levels 5 days after birth (Fig. 1A). In the adult testis, GLUT8 was approximately 40 times more expressed than in the prepubertal testis and was prevalent with respect to GLUT1 (Fig. 1D and E). As expected, T_3 treatment, in the adult testis, did not modify the expression of GLUT1 and 8 (Fig. 1D and E), in fact, at this age, TRs are absent and the testis is unresponsive to thyroid hormones.

Expression and in vivo thyroid hormone regulation of GLUT1

The expression of GLUT1 and GLUT8 in prepubertal Sertoli cells was evaluated by RT-PCR (Fig. 2). Both GLUT mRNAs were expressed in prepubertal Sertoli cells, being, at this stage, GLUT1 prevalent with respect to GLUT8. Figure 2 confirms and quantifies, in prepubertal Sertoli cell primary cultures, the increase of GLUT1 mRNA upon in vitro T_3 treatment (10^{-7} M), detected by RT-PCR analysis. Densitometric analysis of three independent PCR experiments demonstrates the 100% increase in GLUT1 after T_3 treatment (0.65 ± 0.17 vs. 1.23 ± 0.04 AU (arbitrary units), $p < 0.05$; Fig. 2). On the contrary, expression of GLUT8 did

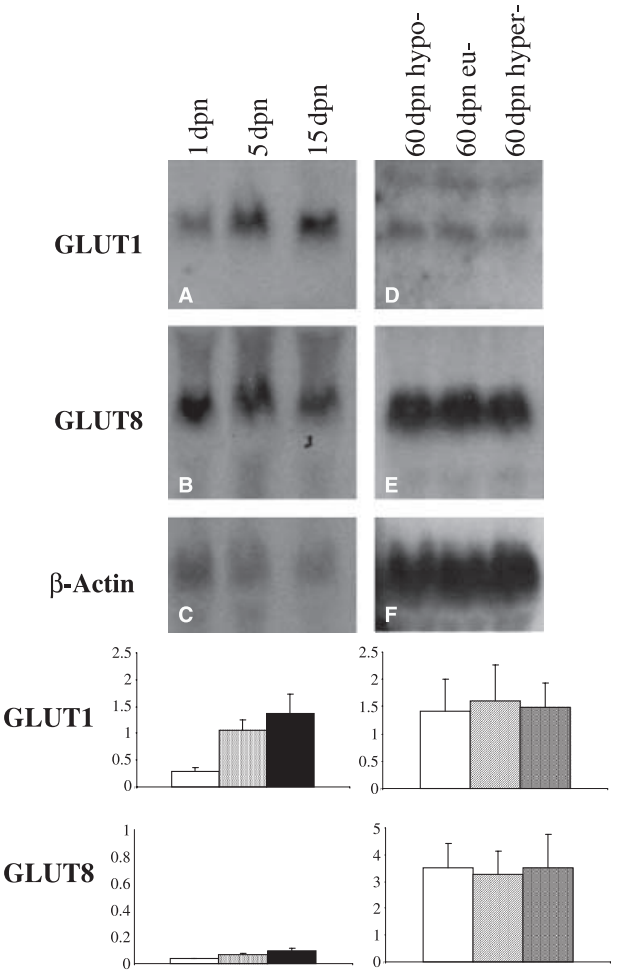

Figure 1. Expression of GLUT1 and GLUT8 mRNA in developing and adult testis. Representative Northern blot analysis of 10 μg/lane of total RNA hybridized with rat GLUT1 and GLUT8 cDNAs. (A) an autoradiograph of RNAs extracted from the whole testis of 1, 5, 15 day *post natum* (dpn) euthyroid rats. Samples were hybridized with GLUT1 probe (A). The film is shown after overnight exposure. The same blot (B) was hybridized with rat GLUT8 probe and exposed for 10 days and then rehybridized with β-actin probe (6-h exposure) (C). (D) A representative autoradiograph of total RNA obtained from the whole testis of adult hypothyroid, euthyroid and hyperthyroid rats hybridized with GLUT1 (6-day exposure); the same blot was hybridized with GLUT8 and exposed overnight (E). It was then normalized with β-actin (3-day exposure) (F). Autoradiographic bands from three separate experiments were scanned using a two-dimensional scanning densitometer and optical densities for each band were obtained. GLUT1 and GLUT8 density were normalized for the optical density obtained by rehybridizing the same filter with β-actin cDNA probe.

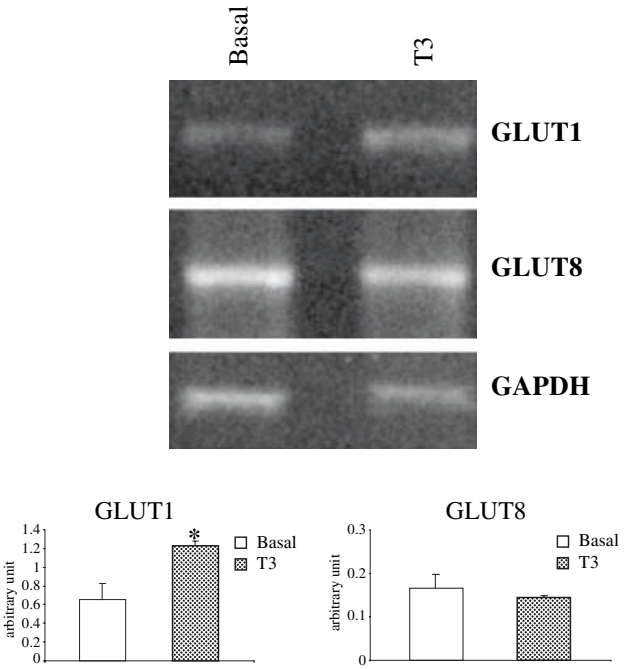

Figure 2. In vitro expression of GLUT1 and GLUT8 mRNAs. Total RNA obtained from prepubertal Sertoli cells before and after T_3 treatment (10^{-7} M; 48 h) was analysed by semiquantitative RT-PCR. The cDNA transcribed from 2.5 μg of total RNA from Sertoli cells was used as a template for PCR amplification of GLUT1, GLUT8. GAPDH was used as loading control of RNA concentration and integrity. Densitometric values represent the mean ± SE of three different experiments; $^*p < 0.05$.

not appear to be under T_3 control. The presence of GLUT8 in prepubertal Sertoli cell and the absence of regulation by T_3 was also confirmed by Northern blot, to avoid the interference of germ cell contamination (Fig. 3).

Action mechanism of GLUT1 regulation by thyroid hormone

To determine whether this effect was caused by augmented transcription induced by GLUT1 promoter, transient transfections were performed with pGL2-GLUT1 in COS7 cell lines in the presence or absence of $TR_{\alpha 1}$, with or without T_3 (Fig. 4). No changes in luciferase expression before and after T_3 treatment were observed at any concentration of T_3 used (from 10^{-6} M to 10^{-9} M; Table 1). Transfection with a vector containing a specific TRE, 3PAL-Luc, was used as a positive control (Fig. 4).

To understand if somatic cells of the seminiferous epithelium have different GLUT1 control mechanisms with respect to other cells, pGL2-GLUT1 was transfected in the adult murine Sertoli cell line TM4 and in the primary culture of immature rat Sertoli cells. As expected, in the TM4 cell lines (Fig. 5), which do not express thyroid receptors, no significant change in luciferase activity was observed in the absence or presence of $TR_{\alpha 1}$ before and after T_3 treatment. In the prepubertal Sertoli cell, no activation of GLUT1 promoter, in either the control or the T_3 treated cell was found (Fig. 5). In the same cells, co-transfection with $TR_{\alpha 1}$ demonstrated activation only in absence of T_3. In contrast, reduction of promoter activity was seen in the presence of T_3.

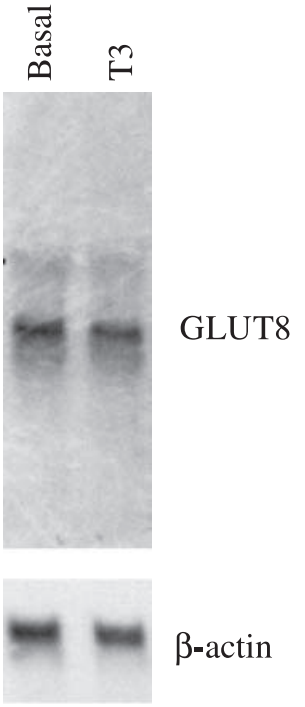

Figure 3. Expression of GLUT8 in prepubertal Sertoli cells before and after T_3 treatment. A 10 μg of total RNA extract of prepubertal Sertoli cells was separed by electrophoresis and hybridized with GLUT8. The concentration and the integrity of RNA was tested with β-actin.

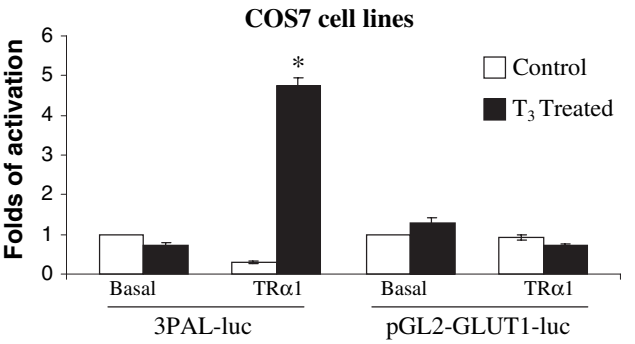

Figure 4. Analysis of rat GLUT1 promoter (pGL2-GLUT1-luc) activity in COS7 cells. The pGL2-GLUT1-luc promoter (see Materials and methods for details) was transfected in the presence or absence of $TR_{\alpha 1}$ and T_3 (10^{-7} M) in COS7 cell lines. Transfection with a vector containing a specific T_3 responsive element, 3PAL-Luc, and $TR_{\alpha 1}$, were used as a positive control. Luciferase activity was measured in the corresponding cell extracts and normalized with respect to *Renilla* luciferase activity. The figure shows the mean ± SD of three determinations; $^*p < 0.05$.

Table 1. Analysis of rat GLUT1 promoter (pGL2-GLUT1) activity in COS7 cells

Treatment (24 h)	Folds of activation (arbitrary units)
pGL2-GLUT1	1
pGL2-GLUT1 + T_3 (10^{-7} M)	0.91 ± 0.11
pGL2-GLUT1 + $TR_{\alpha 1}$	0.89 ± 0.09
pGL2-GLUT1 + $TR_{\alpha 1}$ + T_3 (10^{-9} M)	0.84 ± 0.05
pGL2-GLUT1 + $TR_{\alpha 1}$	0.99 ± 0.08
pGL2-GLUT1 + $TR_{\alpha 1}$ + T_3 (10^{-7} M)	0.64 ± 0.03
pGL2-GLUT1 + $TR_{\alpha 1}$	0.78 ± 0.06
pGL2-GLUT1 + $TR_{\alpha 1}$ + T_3 (10^{-6} M)	0.73 ± 0.05

Thyroid receptor α1 ($TR_{\alpha 1}$); L-triiodothyronine (T_3)

Discussion

We have demonstrated that GLUT types 1 and 8 are both expressed in the testis. However, while GLUT8 is the predominant form in the adult testis, GLUT1 is the main type in the developing testis. GLUT8 expression remains low during development and is high at 60 days. This is in agreement with other findings indicating that GLUT8 is expressed in the acrosomal region of mature spermatozoa, the most abundant cell type in the adult testis (Schurmann *et al.*, 2002). However, we also found GLUT8 present in immature Sertoli cells, although at a lower level than GLUT1. GLUT8 do not appear to be regulated by *in vitro* T_3 treatment in prepubertal Sertoli cells.

We found that GLUT1 follows a different pattern of expression and regulation. In fact, GLUT1 increases during development and reaches adult levels before puberty is completed. In prepubertal Sertoli cells, GLUT1 increases by

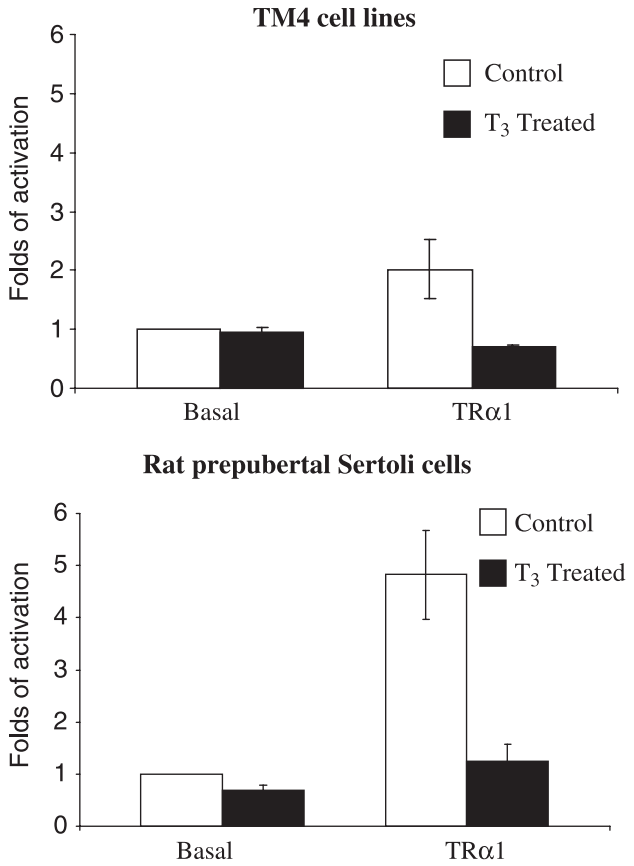

Figure 5. Analysis of rat GLUT1 promoter (pGL2-GLUT1-luc) activity in TM4 and rat prepubertal Sertoli cells. The pGL2-GLUT1-luc promoter (see Materials and methods for details) was transfected in the presence or absence of $TR_{\alpha 1}$ and T_3 (10^{-7} M) in TM4 cell lines and rat prepubertal Sertoli cells. Luciferase activity was measured in the corresponding cell extracts and normalized with respect to *Renilla* luciferase activity. The figure shows the mean ± SD of three determinations; $^*p < 0.05$.

about 100% after T_3 treatment, but does not do so in testes obtained from hyper- and hypothyroid adult animals. This is in agreement with the ontogenetic profile of TR expression in rodents and humans (Jannini *et al.*, 1990, 1994, 1995, 2000). RT-PCR of in vitro treated Sertoli cells confirms (Ulisse *et al.*, 1992) and quantifies for the first time this effect.

The mechanism of action of thyroid hormone stimulation of GLUT1 mRNA is currently unknown. It can be hypothesized that the stimulation of GLUT1 by T_3 may be the result of a nuclear effect through GLUT1 promoter activation. For this reason, we analysed the 5′-flanking region of the GLUT1 gene in various testicular or non-testicular cell lines using transfection experiments. In COS7 cells, thyroid hormone treatment did not modify promoter activity in the presence or absence of the unique T_3-binding TR isoform found in the testis, $TR_{\alpha 1}$ (Jannini *et al.*, 1994).

We then examined the possibility that the effect of T_3 may be mediated by other factors not present in all cells, but only in the testis. For this reason, in transfection experiments we used both primary cultures of cells expressing GLUT1 and $TR_{\alpha 1}$, such as immature Sertoli cells, and a Sertoli cell line derived from an adult murine source, not expressing TRs, such as TM4. In these cells, the GLUT1 promoter was not regulated by T_3 either in presence or absence of $TR_{\alpha 1}$. In fact, the increase the GLUT1 promoter activity, observed in both cell lines only in presence of $TR_{\alpha 1}$ and in absence of T_3, depend on the recruitment of corepressor by unliganded TR and on the subsequent withdrawal of factors from the promoter. Therefore, it merely depends on the excessive presence of co-transfected $TR_{\alpha 1}$ (Tagami *et al.*, 1999).

The possibility that the regulatory region of GLUT1 gene under thyroid hormone control is placed in a different site of the promoter, away from the 2242 bp studied, cannot be currently ruled out. However, at the end of our study, when the complete rat GLUT1 gene sequence became available, we analysed the 5′ promoter flanking region and first intron with sequence scan for transcription factors binding sites and did not find any classical TRE. The result of the in vitro GLUT1 2242 pb promoter analysis, the absence of putative TRE in 5′ flanking region of promoter and first intron of GLUT1 gene and the variable results obtained in different tissues of effect of T_3 stimulation on GLUT1 expression (Weinstein *et al.*, 1991; Weinstein & Haber, 1992; Castello *et al.*, 1994) may support the hypothesis that GLUT1 gene is not the direct target of T_3.

Finally, the increase of GLUT1 after T_3 stimulation in Sertoli cells may be the result of a translation action on mRNA stabilization. In fact, studies from several laboratories have demonstrated that GLUT1 is specifically regulated at post-transcriptional level (Jain *et al.*, 1995; Boado & Pardridge, 1998; Qi & Pekala, 1999) by a group of proteins which can bind a *cis*-acting element located at the 3′-untranslated region of its mRNA. The embryonic lethal abnormal vision (ELAV)/Hu proteins bind to a class of mRNAs containing an AU-rich sequence, stabilizing and/or activating translation of target RNA (Keene, 1999; Brennan & Steitz, 2001). Studies with 3T3-LI adipocyte ectopically expressed Hel-N1 (human ELAV-like neuronal protein 1) demonstrate an increase in GLUT1 protein (eight to ninefolds) and a twofold increase in GLUT1 mRNA (Jain *et al.*, 1997). Expression of ELAV homologue proteins is ubiquitous, and in humans and mice some of them are expressed in the testis (Atasoy *et al.*, 1998). The increase in GLUT1 mRNA observed in Sertoli cells after T_3 treatment may be a consequence of its binding with an ELAV/Hu protein and therefore the major stability of its mRNA. We are currently exploring both these possibilities.

In summary, we confirmed the presence of type 1 and 8 GLUT mRNAs in the rodent testis, and demonstrated their ontogenetic profile. The expression of GLUT1, but not of GLUT8, is increased by the thyroid hormone treatment and this effect is observed only before puberty. Furthermore, we demonstrated that the increase of GLUT1 expression could not be ascribed to a transcriptional action of T_3 on its promoter.

Acknowledgements

The authors are grateful to P. Minelli for her skillful secretarial assistance. We are also indebted to Susanna Dolci for encouragement, Dr Faramarz Ismail-Beigi (Case Western Reserve University, Cleveland, Ohio) for providing pGL2-GLUT1 construct and Ms Marie-Hélène Hayles for adapting her English expertise to our needs. This work was partially supported by the Italian Ministry of Education and the Italian National Research Committee grants.

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Received 6 April 2004; revised 9 June 2004; accepted 15 July 2004