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Follicle-stimulating hormone, testosterone, and hypoxia differentially regulate UDP-glucuronosyltransferase 1 isoforms expression in rat Sertoli and peritubular myoid cells

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Abstract

Uridine diphosphoglucuronosyltransferases (UGTs) are detoxifying enzymes responsible for the metabolism of endogenous and xenobiotics compounds. UGT isoforms are widely distributed in rat tissues showing a constitutive and inducible gene expression. However, little information is available concerning UGTs expression in testis. The UGT1A1, UGT1A2, and UGT1B1 mRNAs expression in whole rat testis, in Sertoli and peritubular myoid cells in basal conditions, and after hormonal and hypoxic stimulation were investigated by reverse transcriptase-polymerase chain reaction (RT-PCR). Constitutive expression of each UGT1 isoform was present in rat testis with higher levels of UGT1A2. UGT transcripts were also detected in Sertoli and peritubular myoid cells. After FSH stimulation, Sertoli cells showed an increase in UGT1B1 mRNA expression, whereas the levels of UGT1A1 and UGT1A2 resulted unmodified. The main effect induced by testosterone was a decrease of UGT1B1 mRNA expression in peritubular myoid cells, whereas in Sertoli cells an increase in UGT1A1 and UGT1B1 was observed. In hypoxic conditions, a reduction in UGTs mRNA levels was detected in both cell types. These findings suggest that rat UGT1 isoforms are regulated in testis by hormonal and environmental factors. Thus, it was speculated that alterations in UGTs expression and/or activity may be involved in the pathogenesis of testis injury.

Keywords: UGT1A1; UGT1A2; UGT1B1; Testis; Hypoxia; FSH

1. Introduction

Uridine diphosphoglucuronosyltransferases (UGTs) are a family of isoenzymes located in the endoplasmic reticulum that catalyse the transfer of the glucuronic acid from uridine diphosphoglucuronic acid to a wide variety of endogenous compounds including bilirubin, steroid hormones, fat-soluble vitamins, and thyroid hormones [1]. UGTs are also involved in the metabolism and inactivation of various xenobiotic compounds such as drugs, teratogens, and carcinogens [2]. Based on their aminoacid sequence similarities, two families of UGTs have been characterized, UGT1 and UGT2, consisting of drug-glucuronidating forms and steroid-glucuronidating forms, respectively. However, it has been demonstrated that UGT1 family is also able to glucoronidate steroids [3,4]. Furthermore, UGT1 family was divided in two subgroups, UGT1A and UGT1B, according to their preferential substrate spe-

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cificity. The members of UGT1 gene complex differ in their first exon but share a single set of commonly used exons (2–5), which encode the common carboxyl terminal of UGT1 isoforms. The variable first exon codes for the N terminus of the enzyme, which determines substrate specificity. In rats, multiple UGT1 isoforms have been identified showing a different tissue distribution and a specific gene expression regulation by xenobiotics and endobiotics. The liver is the main site of expression of most of these enzymes, except for UGT1A7, UGT1A8, UGT1A10, which are expressed only in extrahepatic tissues [5,6]. Furthermore, a direct evidence indicates a widely constitutive and inducible UGT1 expression in several tissues such as brain, lung, intestine, skin, and gonads. Testis has been demonstrated a considerable site of expression of both UGT1 [7] and UGT2 [8,9] families.

In the last decades the rates of testis cancer, cryptorchidism, and hypospadias are increasing [10] while reduction of the sperm counts and male fertility are observed [11]. The reasons of the increased frequency of testicular disorders have been discussed in an intriguing debate. A dozen of synthetic and natural chemicals interfering with the reproductive system, directly or through disruption of endocrine functions, have been discovered. UGTs play a central role in the cellular detoxification of several compounds, preventing accumulation of potentially dangerous xenobiotics or metabolites [12,13] thus avoiding their subsequent bioactivation to even more toxic reactive intermediates. The production of such reactive species can be enhanced by hypoxic conditions, leading to an increased susceptibility to cell injury [14]. The glucuronidation prevents the covalent binding of these compounds to cellular proteins, lipids, and DNA thus hampering cellular damage leading to neoplastic transformation, developmental defects or specific organ diseases.

Although the function and regulation of UGTs are well characterized in several tissues such as liver and kidney, little information is available concerning the relationship between testis and the UGT system.

In the present work, the expression of UGT1A1, UGT1A2, and UGT1B1 isoforms in whole rat testis was tested. Moreover, primary cultures of Sertoli and peritubular myoid cells in basal conditions and after hormonal stimulation were analyzed by reverse transcriptase-polymerase chain reaction (RT-PCR). Finally, the influence of hypoxia on UGT isoforms expression was evaluated.

2. Materials and methods

2.1. Animals

Adult male Sprague–Dawley rats (Charles-River, Como, Italy) 60-days-old were used. After animals were sacrificed by decapitation, testis, liver, kidney and brain were removed, frozen in liquid nitrogen and stored at −70°C until RNA extraction.

2.2. Cell cultures

Sertoli and peritubular myoid cells were isolated from testes of 20-day-old rats by sequential enzymatic digestion with a previously described procedure [15]. Briefly, the testes were aseptically removed, decapsulated and finely minced. Testicular fragments were digested first with 0.25% trypsin (Boehringher Mannheim, Mannheim, Germany) in order to remove interstitial cells and then with 0.1% collagenase (Sigma, St. Louis, MO) in a shaking Dubnoff water bath. Consecutives washes with Dulbecco's modified Eagle's medium (DMEM), with each wash followed by centrifugation at $100 \times g$, separated clusters of Sertoli cells from others testicular cell types. Sertoli cell clusters were resuspended in DMEM/F-12 medium (Gibco-BRL, Paisley, UK) (1:1). Cells were plated in 60 mm culture dishes (Falcon; Becton, Dickinson and Co., Rutherford, NJ) and maintained at 34°C under humidified atmosphere with 5% carbon dioxide. On day 3, plates were treated by hypotonic shock (20 mM Tris–HCl buffer solution, pH 7.4, for 2 min) to remove contaminating germ cells. Contamination with peritubular cells, assessed by staining for alkaline phosphatase, was negligible (1–2%) and contamination with Leidig cells, evaluated by cytochemical detection of 3β-hydroxysteroid dehydrogenase activity, was virtually absent. Viability was assessed by the trypan blue dye exclusion method.

Purified peritubular myoid cells were obtained from the collagenase-digested supernatant, after tubule segment had sedimented, by a Percoll discontinuous gradient technique [16]. Briefly, the collagenase supernatant was layered on the top of the Percoll (Pharmacia, Uppsala, Sweden) gradient (30–70%) and centrifuged at 3000 rpm for 30 min. Then, the band containing purified peritubular myoid cells was separated and the cells were washed and plated. Primary cultures of peritubular myoid cells were maintained at 34°C in 5% CO_2 atmosphere in DMEM/F12 (1:1) containing 10% fetal calf serum (Gibco-BRL). Peritubular myoid cells viability was assessed by the trypan blue exclusion test, and cell purity, never below 98%, was evaluated by alkaline phosphatase activity on adherent cells.

On day 4, Sertoli cells were incubated in the presence of 100 ng/ml ovine FSH (o-FSH-17; NIH Bethesda, MD) or 1 μM testosterone (Sigma), for 24 h. After 6–8 days, peritubular myoid cells were treated with 1 μM testosterone for 24 h in 95% air and 5% carbon dioxide. Other experiments were also performed on Sertoli and peritubular myoid cells in basal conditions placed

in a humidified sealed chamber gassed with 1% O_2, 5% CO_2, 94% N_2 mixture (Caracciolossigeno, Roma, Italy) at 34°C. Gas in and out flow were provided by two steel tubes, which perforated the lid and were connected to two silastic tubings on the outside of the chamber. At the end of the incubation period the tight seal of the chamber was verified by measuring the oxygen tension inside.

After the exposition to hormonal or hypoxic stimuli, Sertoli and peritubular myoid cells were harvested for RNA extraction procedures.

2.3. RT-PCR

The RT-PCR reactions were performed as previously described [17]. Briefly, 1 μg of total RNA extracted from cells with guanidinium isothiocyanate [18], was reverse transcribed, after DNase treatment, in a final volume of 20 μl containing 20 mM Tris HCl (pH 8.3), 50 mM $MgCl_2$, 50 mM KCl, 1 mM each dNTP, 1 U/μl of RNAsin (Gibco-BRL), 100 pmol random hexamer (Boheringer-Mannheim), and 50 U of MuLV reverse transcriptase (Perkin Elmer Cetus, Norwalk, CT) according to the manufacturer's guidelines.

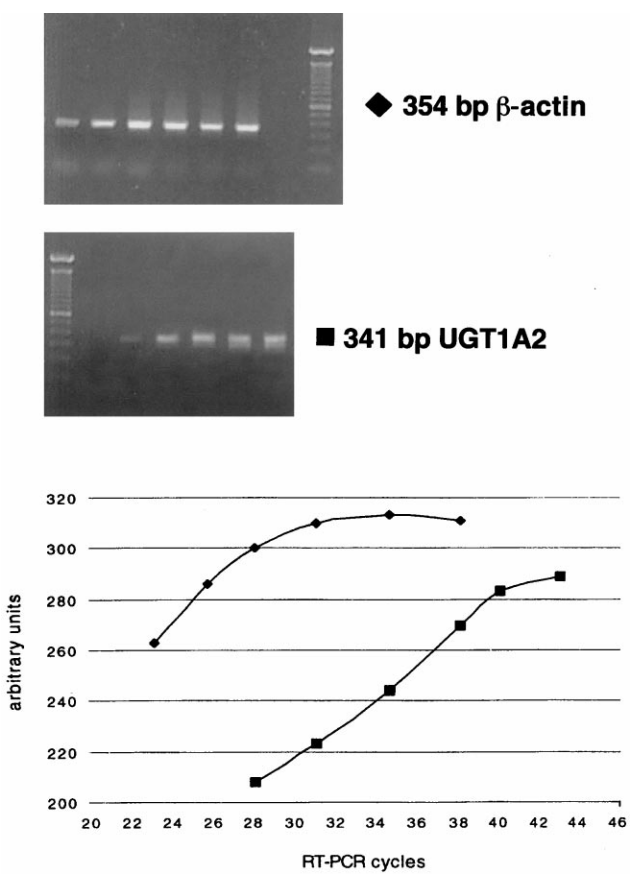

Fig. 1. Kinetics of reverse transcriptase-polymerase chain reaction (RT-PCR) of amplification of UGT1A2 transcripts and human β-actin. *Top*, UGT1A2 and β-actin transcripts in 2% agarose gel. *Bottom*, densitometric quantification of the bands from the agarose gel. Amplification was stopped after 23, 25, 28, 31, 35, or 38 cycles for β-actin, and after 28, 31, 35, 38, 40, or 43 cycles for UGT1A2. Similar results were obtained for UGT1A1 and UGT1B1.

Aliquots of cDNA corresponding to 250 ng of RNA were amplified in PCR buffer containing 25 pmol each of upstream and downstream primers and 1.25 U of *Taq* polymerase (Polymed, Firenze, Italy) in a final volume of 50 μl. Four identical aliquots of the same cDNA were separately amplified with β-actin, UGT1A1, UGT1A2 and UGT1B1 primers in 4 different reactions. The number of amplification cycles, performed in a Perkin Elmer thermocycler 480, were as follows: 37 cycles for UGT1A2, UGT1B1, and UGT1A1, and 26 cycles for β-actin. The number of cycles and the reaction conditions were chosen so that none of the target cDNAs reached a plateau (Fig. 1).

A sample without RNA and a sample without cDNA were included in each experiment of RT-PCR as negative controls. All the recommended precautions were taken in order to avoid contaminations and the preparation of the reaction mixture and analysis of amplified products were carried out in separate rooms. Primers were chosen so as to be located in different exons, in order to prevent DNA amplification.

The sequences of the specific primers were: UGT1A1 upstream: 5′ CCAACGGCTGGCCAACTTCAT 3′ (location 615–632, exon 1); UGT1A2 upstream: 5′ AGTGCCCCAGTCCTCCTTCAT 3′ (location 548–569, exon 1); UGTB1 upstream: 5′ GGGTCACTTGCCACTGAAATC 3′ (location 688–701, exon 1), and a unique downstream primer UGT was used: 5′ GGAGGCGTTGACATAGGCTTC 3′, (location 7–28, exon 2); β-actin upstream: 5′ ACCAACTGGGACGACATGGAG 3′ (location 270–291), downstream: 5′ CGTGAGGATCTTCATGAGGTAAGTC 3′ (location 600–624).

For UGTs amplifications, the PCR cycles were: denaturation step at 94°C for 1 min, annealing step at 60°C for 1 min and primer extension step at 72°C for 1 min and 40 s.

An aliquot of the amplification products was size-fractioned by agarose gel electrophoresis, stained with ethidium bromide and photographed.

To verify the specificity of the amplification products hybridizations with ^{32}P (Amersham, Little Chalfont, UK) were performed. The nucleotide sequences of the probes were: UGT1A1 probe 5′ TATCATTGTCTGTACTCAAAGTATGAGATC 3′ (location 661–691), UGT1A2 probe 5′ CTATCTTGAAACTCACAGATACCATGACTT 3′ (location 581–610), UGT1B1 probe 5′ AACTGCCTTCAGAAAAAAGCCCTATCCCAG 3′ (location 841–871).

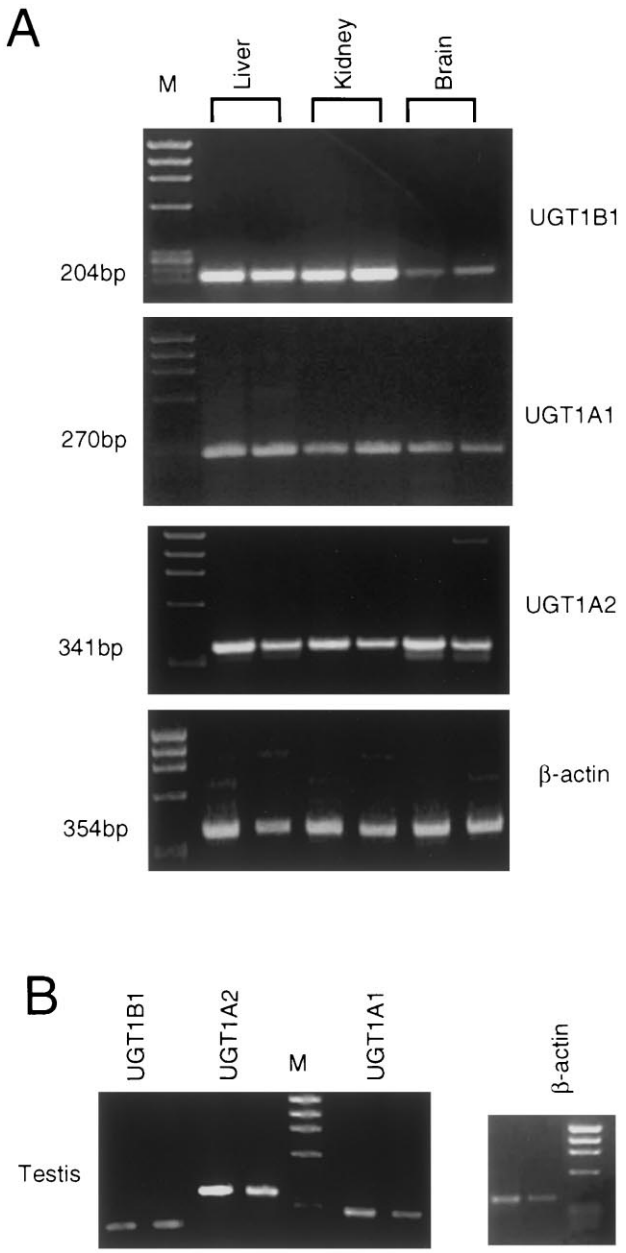

Fig. 2. Expression of uridine diphosphoglucuronosyltransferase (UGT) isoforms and β-actin evaluated by reverse transcriptase-polymerase chain reaction (RT-PCR) in liver, kidney and brain (panel A), and testis (panel B) from two different rats (M = ΦX 174 DNA *Hae*III digested).

The size of the amplified products were 270, 341, 204, 354 bp for UGT1A1, UGT1A2, UGT1B1 and β-actin, respectively.

Furthemore, the identity of the PCR products was verified by direct sequencing.

In order to semi-quantitatively evaluate the amounts of the amplification products a densitometric quantification of the bands from the agarose gel by a densitometric scanner (UMAX) was performed. Values obtained for each UGT isoform were then related to β-actin expression levels in the corresponding sample, determining the UGTs/β-actin ratio. Finally, the modifications in UGTs expression levels comparing the UGTs/β-actin ratio intensity among the different experimental conditions were evaluated.

A statistical analysis of UGTs expression was performed using Anova and Tukey studentized range method (BMDP statistical software, Release 7.0).

3. Results

3.1. Expression of UGT1A1, UGT1A2, UGT1B1 in different rat tissues

The mRNA expression of three different UGT1 isoforms was evaluated in liver, brain, kidney, and testis of adult Sprague–Dawley rats. The expression was analyzed using RT-PCR with a set of primers specific for each UGT1 first exon, the other exons being the same for all UGT1 isoforms. The specificity of the amplified products was verified by direct sequencing and Southern blot hybridization. Constitutive expression of UGT1 isoforms was found in all examined tissues. However, in these experiments a lower expression of UGT1A1 was observed in all tissues. Furthermore, the expression of UGT1B1 was higher in liver and kidney compared to brain and testis, whereas UGT1A2 was the most expressed enzyme in testis as well as in brain (Fig. 2).

3.2. Basal and hormonal regulated UGT1A1, UGT1A2, UGT1B1 mRNA expression in Sertoli and peritubular myoid cells

Sertoli and peritubular myoid cells were obtained from 20-day-old rats. UGT1A1, UGT1A2, UGT1B1 mRNA expression was detected in basal conditions both in Sertoli and peritubular myoid cells. Then, UGT isoforms expression was evaluated in Sertoli and peritubular cell cultures treated with 100 ng/ml FSH and 1 μM testosterone for 24 h. The levels of UGTs mRNA expression was modified by the administration of specific hormonal stimuli (Table 1). In the presence of FSH, Sertoli cells showed an increase in UGT1B1 mRNA expression, whereas the transcriptional levels of UGT1A1 and UGT2A2 resulted unmodified (Fig. 3). Testosterone administration to Sertoli cells induced a slight increase of UGT1A1 and UGT1B1 mRNA levels while no difference was detected for UGT1A2 expression.

Changes in UGT1A1 and UGT1B1 mRNA expression became detectable during stimulation of peritubular myoid cells with testosterone. In fact, UGT1A1 mRNA expression was increased, while a decrease of UGT1B1 mRNA level was observed. No modification of UGT1A2 mRNA level was detected (Fig. 3).

3.3. Effect of hypoxia on UGT isoform mRNAs expression in Sertoli and peritubular myoid cells

UGT1A1, UGT1A2, UGT1B1 mRNA expression in Sertoli and peritubular myoid cells was evaluated after exposure of primary cultures to premixed gases containing 1% O_2, 5% CO_2, balance N_2 for 24 h. A general tendency to a decrease of UGT isoform mRNA levels was observed after hypoxic exposure. Particularly, Sertoli cells showed a prevalent reduction in UGT1A2 mRNA expression, while UGT1B1 isoform was the most inhibited in peritubular myoid cells (Fig. 3) (Table 1).

4. Discussion

The results, in agreement with previous reports showing testis as a considerable site of expression of UGT isoforms, demonstrate that UGT1A1, UGT1A2, and UGT1B1 are constitutively expressed in the male rat gonad. Furthermore, among UGT isoforms studied, UGT1A2 is the most expressed in rat testis as well as in brain.

Although the regulation of UGTs by endocrine substances has been less extensively studied than the modulatory effect of xenobiotic compounds, some authors have demonstrated that hormones differentially regu-

Table 1
Uridine diphosphoglucuronosyltransferase (UGT) and β-actin mRNA isoforms expression ratio evaluated by densitometric analysis of reverse transcriptase-polymerase chain reaction (RT-PCR) products in rat Sertoli and Peritubular myoid cells[a]

Cells	UGT1B1/β-actin	UGT1A1/β-actin	UGT1A2/β-actin
Sertoli	0.43 ± 0.07	0.38 ± 0.07	0.46 ± 0.07
Sertoli hypoxia	0.31 ± 0.09**	0.27 ± 0.06*	0.24 ± 0.06**
Sertoli + FSH	0.77 ± 0.07**	0.38 ± 0.08 (n.s.)	0.45 ± 0.07 (n.s.)
Sertoli + testosterone	0.59 ± 0.08**	0.50 ± 0.10*	0.48 ± 0.06 (n.s.)
Peritubular	0.53 ± 0.06	0.43 ± 0.07	0.44 ± 0.06
Peritubular hypoxia	0.37 ± 0.10**	0.47 ± 0.08 (n.s.)	0.33 ± 0.06**
Peritubular + testosterone	0.28 ± 0.07**	0.58 ± 0.07**	0.40 ± 0.06 (n.s.)

[a] The results are the mean of three independent experiments, each one in triplicate ($x \pm \sigma$). Statistical analysis was performed by Anova and Tukey studentized range method (BMDP statistical software, Release 7.0). n.s., not significant.

* $P < 0.05$.

** $P < 0.01$.

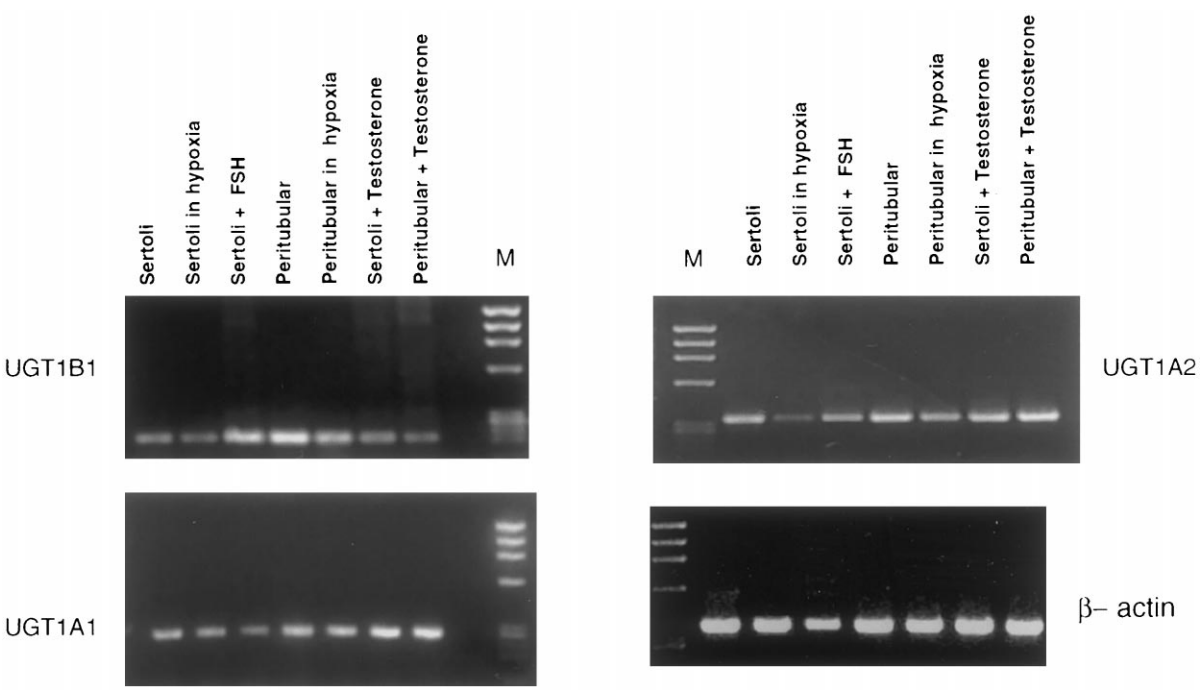

Fig. 3. Reverse transcriptase-polymerase chain reaction (RT-PCR) analysis of UGT1B1, UGT1A1, UGT1A2 and β-actin gene expression in Sertoli and peritubular myoid cells. Sertoli and peritubular cells are shown in cultural basal condition, in hypoxic condition, and after hormonal stimulation (M = ΦX 174 DNA *Hae*III digested).

late the expression of several UGT isoforms. In fact, recent studies showed a feedback regulation of UGT1 family by iodothyronines in response to metabolic changes in thyroid status [19,20]. Sex hormones differentially regulate UGT1 and UGT2 isoforms. Marked differences in liver UGT isoforms levels have been detected between male and female rats with significant alterations after gonadectomy and hormonal replacement [21]; moreover progesterone and oestradiol up regulate, together with hCG, the level of phenol UGT in rat ovary [22]. Glucocorticoids are able to stimulate UGTs expression and activity [23]. Finally, besides hCG, hypophyseal hormones such as growth hormone modify UGTs expression [24]. These reports prompted us to investigate whether the UGT1A1, UGT1A2, and UGT1B1 gene expression is under hormonal control. mRNA levels of these isoforms where evaluated in basal condition, and after hormonal treatment with FSH and testosterone in rat primary cultures of Sertoli and peritubular myoid cells. Activity of both cell types is dependent on hypophyseal hormones: FSH modulates Sertoli cells in a direct manner, while LH regulates peritubular myoid cell function through testosterone production by Leydig cells. The experiments showed that UGT transcripts are constitutively expressed in Sertoli and peritubular myoid cells and that hormonal treatment can modify those levels. Testosterone and FSH treatment upregulates the UGTB1 expression in Sertoli cells, while the same gene is downregulated by testosterone treatment in peritubular myoid cells. The UGT1A1 and UGT1A2 expression is not significantly affected by FSH treatment, while testosterone modifies the UGT1A1 expression both in Sertoli and peritubular myoid cells. UGT1A2 was not affected by hormonal treatment.

As previously shown, hypoxia reduces the ability of cells to tolerate damage from reactive species increasing their susceptibility to injury. Some reports have described a negative effect of hypoxia on UGTs activity [25,26] without exploring any potential effect at transcriptional level. The results show that hypoxic conditions decrease the UGT1B1 and UGT1A2 mRNA levels either in Sertoli and peritubular cells. On the other hand, hypoxia does not affect or slightly modifies the UGTIA1 expression in peritubular and Sertoli cells, respectively. In view of these results, it can be hypothesized that pathologic conditions like varicocele, thickness of tubular basal membrane, damage of periferic circulation, inducing a decrease in oxygen tension in testis can modify the UGTs expression and therefore reduce the ability of such enzymes to inactivate potential damaging substances.

The results, suggest that rat UGT1 genes can be individually regulated in testis by hormonal and environmental factors, i.e. hypoxic conditions.

These preliminary data lead to the speculation that alterations in UGTs expression and/or activity due to toxic environmental exposure may be involved in the pathogenetic mechanisms responsible for testis injury. Further studies would be required to better understand the potential role of UGT enzyme in testis pathology.

References

[1] P.I. Mackenzie, I.S. Owens, B. Burchell, K.W. Bock, A. Bairoch, A. Belanger, S. Fournel-Gigleux, M. Green, D.W. Hum, T. Inayagi, D. Lancet, P. Louisot, J. Magdalou, J. Roy Chowdhury, J.K. Ritter, H. Schachter, T.R. Tephly, K.F. Tipton, D.W. Nerbert, The UDP glycosyltransferase gene superfamily: recommended nomenclature update based on evolutionary divergence, Pharmacogenetics 7 (4) (1997) 255–269.

[2] S.B. Senafi, D.J. Clarke, B. Burchell, Investigation of the substrate specificity of a cloned expressed human bilirubin UDP-glucuronosyltransferase: UDP-sugar specificity and involvement in steroid and xenobiotic glucuronidation, Biochem. J. 303 (1994) 233–240.

[3] C.A. Albert, M. Vallee, G. Beaudry, A. Belanger, D.W. Hum, The monkey and human uridine diphosphate-glucuronosyltransferase UGT1A9, expressed in steroid target tissues, are estrogen-conjugating enzymes, Endocrinology 140 (7) (1999) 2392–3302.

[4] C.D. King, M.D. Green, G.R. Rios, B.L. Coffmann, I.S. Owens, W.P. Bishop, T.R. Tephly, The glucuronidation of exogenous and endogenous compounds by stably expressed rat and human UDP-glucuronosyltransferase 1.1, Arch. Biochem. Biophys. 332 (1996) 92–100.

[5] C.P. Strassburg, K. Oldhafer, M.P. Manns, R.H. Tukey, Differential expression of the UGT1A1 locus in human liver, biliary, and gastric tissue: identification of UGT1A10 transcripts in extrahepatic tissue, Mol. Pharmacol. 52 (1997) 212–220.

[6] C.P. Strassburg, M.P. Manns, R.H. Tukey, Expression of the UDP-glucoronosyltransferase 1A locus in human colon. Identification and characterization of the novel extrahepatic UGT1A1. J. Biol. Chem. 273 (1998) 8719–8726.

[7] P.A. Munzel, M. Bruck, K.W. Bock, Tissue-specific constitutive and inducible expression of rat phenol UDP-glucuronosyltransferase, Biochem. Pharmacol. 47 (1994) 1445–1448.

[8] F. Chen, J.K. Ritter, M.G. Wang, W. Mc Bride, R.A. Lubet, I.S. Owens, Characterization of a cloned human dihydrotestosterone/androstanediol UDP-glucuronosyltransferase and its comparison to other steroid isoforms, Biochemistry 32 (1993) 10648–10657.

[9] M. Beaulieu, E. Levesque, O. Barbier, D. Turgeon, G. Belanger, D.W. Hum, A. Belanger, Isolation and characterization of a simiam UDP-glucuronosyltransferase UGT2B18 active on 3-hydroxyandrogens, J. Mol. Biol. 275 (1998) 5785–5794.

[10] R.M. Sharpe, N.E. Skakkebaek, Are oestrogens involved in falling sperm counts and disorders of the male reproductive tract?, The Lancet 341 (1993) 1392–1395.

[11] E. Carlsen, A. Giwercman, N. Keiding, N.E. Skakkebaek, Evidence for decreasing quality of semen during past 50 years, Br. Med. J. 305 (1992) 609–613.

[12] C.H. Brierley, B. Burchell, UDP-glucuronosyltransferases, chemical defense, jaundice and gene therapy, BioEssay 15 (1993) 749–754.

[13] D.S. Vienneau, U. DeBoni, P.G. Wells, Potential genoprotective role for UDP-glucuronosyltransferase in chemical carcinogenesis: initiation of micronuclei by benzo(a)pyrene and benzo(e)pyrene in UDP-glucuronosyltransferase-deficient cultured rat skin fibroblasts, Cancer Res. 55 (1995) 1045–1051.

[14] X. Shan, T.Y. Aw, E.R. Smith, M. Ingelman-Sundberg, B. Mannervik, T. Iyanagi, D.P. Jones, Effect on chronic hypoxia on

detoxication enzymes in rat liver, Biochem. Pharmacol. 43 (1992) 2421–2426.
[15] V. Santiemma, F. Beligotti, M. Magnanti, S. Palleschi, L. Silvestroni, A. Fabbrini, Endothelin-1 stimulates deoxyribonucleic acid synthesis and contraction in testicular peritubular myoid cells, Biol. Reprod. 54 (1996) 583–589.
[16] P.S. Tung, I.B. Fritz, Transforming growth factor-β and platelet-derived growth factor synergistically stimulate contraction by testicular peritubular cells in culture in serum-free medium, J. Cell. Physiol. 46 (1991) 386–393.
[17] P. Gazzaniga, A. Gradilone, R. Vercillo, O. Gandini, I. Silvestri, M. Napolitano, L. Albonici, A. Vincenzoni, M. Gallucci, L. Frati, A.M. Aglianò, *bcl 2/bax* mRNA expression ratio as prognostic factor in low grade urinary bladder cancer, Int. J. Cancer 69 (1996) 100–104.
[18] P. Chomczynski, N. Sacchi, Single-step method of RNA isolation by acid guanidium thiocyanate-pheno-chloroform extraction, Anal. Biochem. 162 (1987) 156–159.
[19] T. Masmoudi, A.K. Hihi, M. Vazquez, A.Y. Desvergne, W. Wahli, H. Goudonnet, Transcription regulation by triiodothyronine of the UDP-glucoronosyltransferase family 1 gene complex in rat liver, J. Biol. Chem. 27 (1997a) 17171–17175.
[20] T. Masmoudi, J. Mounie, Y. Artur, J. Magdalou, H. Goudonnet, Comparative quantification of two hepatic UDP-glucuronosyltransferase bilirubin isoforms mRNAs in various thyroid states in rat, Biochem. Pharmacol. 53 (1997b) 1013–1017.
[21] S.I. Strasser, S.A. Smid, M.L. Mashford, P.V. Desmond, Sex hormones differentially regulate isoforms of UDP-glucuronosyltransferase, Pharmacol. Res. 14 (1997) 1115–1121.
[22] L. Becedas, B. Lundgren, J.W. De Pierre, Characterization of the UDP-glucuronosyltransferase isoenzyme expressed in rat ovary and its regulation by gonadotropins. Biochem. J. 332 (1998) 51–55.
[23] Y. Emi, S. Ikushiro, T. Iyanagi, Drug-responsive and tissue-specific alternative expression of multiple first exons in rat UDP-glucuronosyltransferase family 1 (UGT1) gene complex, J. Biochem. (Tokio) 117 (1995) 392–399.
[24] F. Gueraud, T. Masmoudi, H. Goudonnet, A. Paris, Differrential effect of hypophysectomy and growth hormone treatment on hepatic glucuronosyltransferases in male rats: evidence for an action at a pretranslational level for isoforms glucuronidating bilirubin, Biochem. Pharmacol. 53 (1997) 1637–1647.
[25] T.Y. Aw, D.P. Jones, Secondary bioenergetic hypoxia, J. Biol. Chem. 15 (1982) 8997–9004.
[26] T.Y. Aw, X.Q. Shan, A.H. Sillau, D.P. Jones, Effect of chronic hypoxia on acetaminophen in the rat, Biochem. Pharmacol. 42 (1991) 1029–1038.