The Effect of Hormones on the Expression of Five Isoforms of UDP-Glucuronosyltransferase in Primary Cultures of Rat Hepatocytes

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Purpose. To investigate the direct effects of sex hormones, growth hormone, thyroid hormones and dexamethasone on the regulation of UDP-glucuronosyltransferase (UGT).

Methods. Rat hepatocytes were cultured on matrigel and treated with various hormones. Northern blot analysis was carried out using cDNA probes to family 1 and family 2 isoforms.

Results. Treatment with 10^{-5} M testosterone increased the mRNA levels of UGT 2B1 by 29% and UGT2B3 by 32%. Incubation of growth hormone (10 mU) with hepatocytes suppressed the expression of UGT2B1 and UGT2B3 by 17% and 38%, respectively. T3 administration resulted in a time and dose-dependent effect on the expression of UGT 1 isoforms, with increased UGT1A6 by 70%, and decreased UGT1A1 by 38% and UGT1A5 by 35%. All UGT isoforms except UGT 1A6 studied in this assay were up-regulated by dexamethasone, but to different degrees. The regulation of UGT1A1 and UGT2B1 by dexamethasone was dose and time dependent, and the induction of dexamethasone in the expression of UGT1A1 and UGT2B1 was blocked by cycloheximide but not dichloro-1-D-ribofuranosylbenzimidazole.

Conclusions. This study demonstrates that multiple hormones take part in the regulation of UGT mRNA expression in the rat and individual genes can be differentially modulated.

KEY WORDS: UDP-glucuronosyltransferase; sex hormones; growth hormone; thyroid hormones; dexamethasone.

INTRODUCTION

UDP-glucuronosyltransferase (UGT) is a superfamily of enzymes that catalyze the conjugation of glucuronic acid to both endogenous compounds including bilirubin, bile acids, steroid and thyroid hormones and to exogenous compounds including food additives, therapeutic drugs and environmental pollutants (1). Based on nucleotide and amino acid sequence differences, mammalian UGT can be grouped into three major families. The gene for UGT family 1 including a bilirubin cluster (UGT1A1 and UGT 1A5) and a phenol cluster (UGT 1A6) are located on chromosome 2q37. These enzymes are formed by alternative splicing of an isoform specific exon encoding a unique N-terminal region with common exons 2 to 5 encoding an identical C-terminal region. UGT family 2 isoforms are each derived from an individual gene. UGT family 8 has only one member (1).

Previously whole animal studies have demonstrated that gonadal hormones moderate hepatic metabolism of both endogenous and exogenous compounds. Testosterone treatment increases the glucuronidation of p-nitrophenol but decreases that of bilirubin and estrogen (2,3). Progesterone on the other hand increases bilirubin conjugation and addition of estradiol further increases bilirubin glucuronidation (3). More recently, we reported that gonadectomy in male rats resulted in reduced expression of mRNAs for UGT2B1 and UGT2B3. Testosterone replacement was associated with higher messenger RNA (mRNA) levels than that seen in controls (4). Thyroid hormones increase the expression of UGT 1A2 and decrease the expression of UGT1A6 whereas dexamethasone selectively up-regulates the expression of UGT 1 family isoenzymes which conjugate bilirubin (5,6).

In view of the known complex interrelationship of hormones involving the pituitary, adrenal glands, gonads, and other organs, it is very difficult to ascribe any particular effect in the whole animal to a single hormone. The involvement of hormonal factors in UGT regulation is thus mainly inferred from indirect evidence. A demonstration of their direct participation in the control of UGT gene expression may add significantly to our understanding of UGT regulation.

Primary hepatocyte culture offers a useful model system in which exposure of liver cells to hormones can be precisely controlled and the effect of individual hormones delineated. The present study was designed to use primary cultured hepatocytes to investigate the roles of sex hormones, growth hormone, thyroid hormones and dexamethasone in the regulation of three isoforms of UGT1 family and two isoforms of UGT2 family.

MATERIALS AND METHODS

Materials

Adult male Sprague-Dawley rats (Biological Research Laboratories, Austin Hospital, Melbourne), weighing 200–250 g, were maintained under standard conditions of light and temperature, with free access to animal chow and water. These experiments were approved by the St. Vincent's Hospital Animal Ethics Committee and the research adhered to the "NIH Principles of Laboratory Animal Care."

Recombinant human growth hormone was obtained from Pharmacia (AB, Sweden). Matrigel was purchased from the Storr Liver Unit, Westmead Hospital, Australia. Collagenase (type IV), Cell culture medium (Waymouth MB 752/1), cycloheximide, 5,6-dichloro-1-D-ribofuranosylbenzimidazole (DRB), L-triiodothyronine (T3), thyroxine (T4), dexamethasone, testosterone, dihydrotestosterone, estradiol and progesterone were purchased from Sigma Chemical Co.(St. Louis. MO)

Primary Culture of Rat Hepatocytes

The hepatocytes were prepared by *in situ* collagenase perfusion through the portal vein of phenobarbitone-anesthetized

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ABBREVIATIONS: UGT, UDP-glucuronosyltransferase; mRNA, messenger RNA; T3, L-triiodothyronine; T4, thyroxine; DRB, 5,6-dichloro-1-D-ribofuranosylbenzimidazole; SDS, sodium dodecyl sulfate; SSPE, 0.15M NaCl, 0.01M NaH_2PO_4, 1 mM ethylenediaminetetraacetic acid; cDNA, complementary DNA; rRNA, ribosomal RNA.

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rats based on the method of Bissell and Guzelian (7). Cell viability was 85–95% determined by Trypan blue exclusion. Matrigel (200 μl) was evenly inoculated onto 60-mm surface modified polystyrene petri dishes (Becton Dickinson, New Jersey, USA) and allowed to form a gel at room temperature before cell isolation. To each petri dish was added at 3×10^6 isolated hepatocytes suspended in 3 ml of a Modified Waymouth MB752/1 medium, to which $NaHCO_3$ (2.24 g/l), Na_2SeO_3 (0.0173 mg/l), $ZnSO_4.7H_2O$ (1.47 mg/l), L-ornithine.1HCl (20 mg/l), ascorbic acid (36.4 mg/l), and penicillin/streptomycin (60.2 mg/l) were added at the indicated concentrations. Insulin (25 units/l) was the only hormone added to the Waymouth MB 752/1 medium. Cultures were maintained in a humidified incubator at 37°C in an atmosphere containing 5% CO_2. The medium was renewed at 4 h and 20 h and every 24 h thereafter to remove non-attached, non-viable cells. Hormones were added to the medium on the fourth day of cell culture, and subsequent changes of medium every 24 h included the test hormones until the cells were harvested.

Hepatocytes were harvested according to the method of Liddle (8). The medium was poured off carefully and the cells washed three times in cold phosphate-buffered saline. Two ml of ice-cold phosphate-buffered saline containing 5 mM ethylenediaminetetraacetic acid, pH 7.4, was then added. The plates were kept at 4°C for 25–30 min, then cells and matrigel were removed by scraping with a Teflon-coated scraper and transferred into a 50 ml sterile Falcon tube. They were then allowed to stand on ice for 15–20 min to allow the matrigel to be fully dissolved in the buffer. The cells were collected by centrifugation (50 g for 1 min) and washed three times in PBS. The hepatocytes were stored at −70°C until required.

RNA Extractions

RNA was extracted from cultured hepatocytes by the guanidinium thiocyanate-phenol-chloroform procedure as described previously (9). The pellet was washed with 75% ethanol, dissolved in diethylpyrocarbonate-treated water, and stored at −70°C. The total RNA obtained was quantified by spectrophotometry at 260nm. Sample purity was determined by calculating the ratio of sample absorbency at 260:280 nm, which was normally 1.7–2.0.

Northern Blot Hybridization

RNA (20 μg) was denatured by heating for 15 min at 65°C, and then electrophoresed on a 1.5% formaldehyde agarose gel, transferred to Hybond-N nylon filters (Amersham, Buckinghamshire, U.K.) in 20 × standard saline citrate overnight. RNA was cross-linked by UV irradiation. Prehybridization was performed for 3–4 h at 42°C in 50% formamide, 0.5% polyvinylpyrrolidone, 0.5% Ficoll, 0.5% bovine serum albumin (fraction V), 1% skim milk powder, 0.5% SDS (sodium dodecyl sulfate), 5 × SSPE (0.15M NaCl, 0.01M NaH_2PO_4, 1 mM ethylenediaminetetraacetic acid). Hybridization was then performed by adding to the prehybridization solution the appropriate complementary DNA (cDNA) probes labelled with α-^{32}P-dCTP by random priming (Dupont, Sydney, Australia). Incubation was continued overnight. Filters were then washed in 2 × SSPE/0.1%SDS for 15 min at 42°C, followed by 20-min wash in 1 × SSPE/0.1%SDS at 37°C and 5-min wash in 0.1 × SSPE/0.1%SDS at room temperature. Quantitation of mRNA was by scanning of the phosphorimager screen using the Imagequant program (Molecular Dynamic). Membranes were rehybridized with an 18s ribosomal RNA (rRNA) oligo probe to allow correction for difference in RNA loading.

Probes consisting of 913 base pair, 476 base pair and 1034 base pair cDNA fragments corresponding respectively to exon B1(UGT1A1), B5(UGT1A5) and A1(UGT1A6) of UGT 1 gene (6), were kindly provided by Dr Takashi Iyanagi of Himeji Institute of Technology, Akogun Hyogo, Japan. Full-length rat UGT 2B1 (10) and UGT 2B3 cDNA (11) probes were generously provided by Dr Peter Mackenzie (Flinders Medical Center, Bedford Park, South Australia). A 30-mer oligo probe (CGG CAT GTA TTA GCT CTA GAA TTA CCA CAG) to 18s rRNA was synthesized by Bresatec, Thebarton, South Australia.

Measurement of mRNA Stability

The effect of dexamethasone on RNA stability was studied in hepatocyte cultures treated with DRB to inhibit new RNA synthesis. Hepatocytes were incubated with control medium or with medium containing dexamethasone (10^{-6} M) for 24 h. Incubation was then continued after addition of DRB 25 μg/ml. Hepatocytes were harvested at 0, 6, 12, 18, 24 h after addition of DRB. Northern blot analysis was performed to determine mRNA levels of UGT1A1 and UGT2B1.

Data Analysis

Experimental data are presented as means ± standard error. Statistical significance was determined according to the Students t-test using sigma stat (Jandel Scientific Software, San Rafael, CA).

RESULTS

In preliminary experiments, the expression of most UGT mRNAs in hepatocytes decreased by 30–40% over the first three days. However, by day four, the levels of these UGT mRNAs recovered to 40–50% above the level in freshly isolated cells, and were then relatively constant (Fig. 1). Expression of UGT1A6 mRNA differed in manifesting a slight increase until

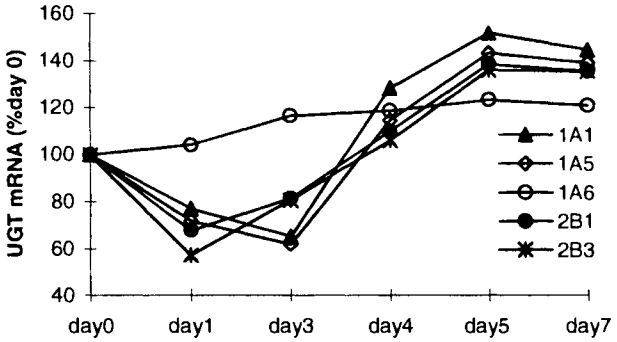

Fig. 1. Expression of mRNAs for UGT1A1, UGT1A5, UGT1A6, UGT2B1 and UGT2B3 in primary cultures of rat hepatocytes harvested over 7 days. The freshly isolated hepatocyte values (day 0) are set at 100%, other values within the same experiment are presented as a percentage of freshly isolated hepatocyte values. Each experimental point represents the average of UGT mRNA concentrations normalized against 18s rRNA in two separate experiments.

day four and then remaining at that level to day seven of culture (Fig. 1). These results suggest that at least three days are required before full recovery of expression of UGT in cultured hepatocytes. Therefore, in the following experiments, hepatocytes were cultured for three days prior to treatment with the various hormones. Both concentrations of hormones added and lengths of treatment were as determined by dose-dependent and time course studies (data not shown for sex hormones and growth hormone).

Effect of Sex Hormones

Treatment of hepatocytes for 48 h with 10^{-5} M testosterone increased the mRNA levels of both UGT 2B1 and UGT 2B3 by 129% and 132% of control, respectively. In contrast, little change was found in the mRNA levels of three isoforms of the UGT 1 family after incubation of hepatocytes with testosterone. Both UGT 2B1 and UGT 2B3 mRNA levels also showed increases after treatment with dihydrotestosterone, but these did not reach significance (Table 1).

Neither estradiol nor progesterone at concentration of 10^{-5} M resulted in appreciable change in mRNA levels corresponding to either family 1 or family 2 UGT genes (Table 1).

Effects of Growth Hormone

Addition of growth hormone at the concentration of 10 mU to the culture medium for 24 h resulted in a decrease in the expression of UGT 2B1 mRNA (83% of control), and UGT 2B3 mRNA (62% of control). The expression of UGT 1A1, UGT1A5 and UGT1A6 mRNAs was not significantly modified when growth hormone at the concentration of 10 mU was used (Table 1).

Effects of Thyroid Hormones

The addition of T3 at the concentration of 10^{-8} M for 24 h to the medium increased UGT1A6 mRNA level by 170% of control whereas the expression of UGT1A1 and UGT1A5 mRNA levels were reduced by 62% and 65% of control, respectively. No detectable change was observed in UGT 2B1 and UGT 2B3 mRNA levels (Table 1). Clear changes were observed 12 h after the addition of T3 and were maximal at 24 h for UGT1A1, UGT1A5 and UGT1A6 mRNAs after the initiation of hormone exposure (Fig. 2).

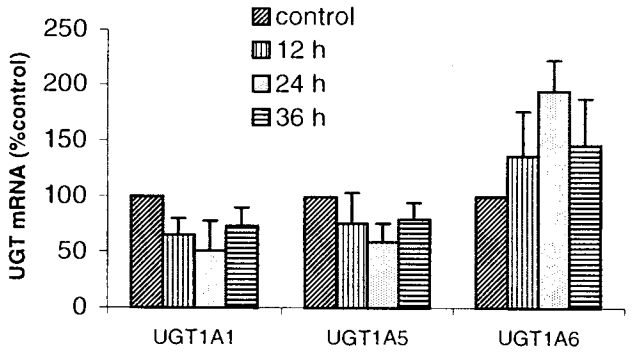

Fig. 2. Time course of UGT1A1, UGT1A5 and 1A6 mRNA levels in cultured hepatocytes. Hepatocytes at day 4 were incubated with L-triiodothyronine (T3 10^{-8} M). Cells were harvested for RNA preparation at different time points. The mean of UGT mRNA concentrations normalized against 18s rRNA in two separate experiments are shown. Data are expressed as percentage of control values on the indicated times.

The addition of T4, at final concentrations of 10^{-7} M for 24 h, had little effect on the expression of five UGT mRNAs (Table 1).

Effects of Dexamethasone

At a concentration of 10^{-6} M, dexamethasone significantly increased mRNA levels for UGT1A1, UGT1A5, UGT2B1 and UGT2B3. No effect was seen on mRNA level for UGT1A6 (Table 1). Detectable increases of UGT1A1 and UGT2B1 mRNAs were noted at dexamethasone concentrations down to 10^{-9} M (Fig. 3). At the concentration of 10^{-6} M, significant increases in mRNA levels for UGT1A1 and UGT2B1 were observed 12 h after the addition of dexamethasone and these peaked at 24 h (Fig. 4).

Block of protein synthesis by the inhibitor, cycloheximide, at the concentration of 10 μg/ml, prevented the induction of UGT1A1 and UGT2B1 mRNAs by dexamethasone in cultured hepatocytes, at both 12 h and 24 h (Fig. 5). The addition of DRB (25 μg/ml) to block transcription, did not affect the rate

Table 1. Effect of Hormones on mRNA Levels for UGT1A1, UGT1A5, UGT1A6, UGT2B1, and UGT2B3 in Cultured Rat Hepatocytes

	UGT1A1 mRNA	UGT1A5 mRNA	UGT1A6 mRNA	UGT2B1 mRNA	UGT2B3 mRNA
Oestradiol (10^{-5} M)	123 ± 21.3	101 ± 5.9	111 ± 14.5	125 ± 23.2	114 ± 13.1
Progesterone (10^{-5} M)	120 ± 16.0	116 ± 26.6	99 ± 12.9	113 ± 13.2	94 ± 12.9
Testosterone (10^{-5} M)	113 ± 19.8	127 ± 20.6	92 ± 6.6	129 ± 10.1*	132 ± 9.6*
Dihydrotestosterone (10^{-5} M)	104 ± 20.9	121 ± 21.9	97 ± 7.3	121 ± 12.3	125 ± 20.2
Growth Hormone (10 mU)	117 ± 16.0	104 ± 20.5	87 ± 13.9	83 ± 8.2*	67 ± 9.7*
L-triiodothyronine (10^{-8} M)	62 ± 5.4*	65 ± 11.8*	170 ± 16.4*	96 ± 9.7	101 ± 13.6
Thyroxine (10^{-7} M)	92 ± 3.5	89 ± 12.5	112 ± 24.2	104 ± 15.5	115 ± 16.2
Dexamethasone (10^{-6} M)	316 ± 86*	187 ± 50*	97 ± 10.6	171 ± 23*	165 ± 33*

Note: Hepatocytes at day 4 were incubated with hormones in the concentrations indicated below and cells were harvested 24 h later for growth hormone, thyroid hormone and dexamethasone treatment groups, and 48 h later for sex hormone treatment group (The values are presented as percentage of control ± standard error in three individual experiments.* $p < 0.05$).

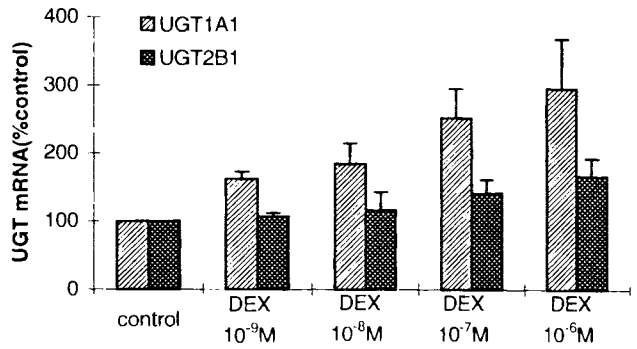

Fig. 3. Dose-dependent effect of dexamethasone on UGT1A1 and UGT2B1 mRNA expression in cultured rat hepatocytes. Hepatocytes at day 4 were incubated with dexamethasone in the concentrations indicated (DEX 10^{-9} M–10^{-6} M) and cells were harvested for RNA preparation 24 h later. The mean of UGT mRNA concentrations normalized against 18s rRNA in two separate experiments are shown. The day 4 control values are set at 100%, other values within the same experiment are presented as a percentage of control value.

of decline of mRNA concentration of either control or dexamethasone (10^{-6} M) treated preparations for either UGT1A1 or UGT2B1 (Fig. 6).

DISCUSSION

Primary hepatocyte cultures have been widely used to study the hormonal regulation of cytochrome P450 enzyme activities and gene expression. They permit the control of hormone concentrations and permit analysis of the early results associated with hormone-receptor interaction with regulatory elements. Recently, Emi et al reported that both UGT1A1 and UGT1A6 mRNAs were expressed in primary cultured hepatocytes, and that 3-methylcholanthrene induced UGT1A6 mRNA, consistent with its activity in intact rats (12). In the present study we have further demonstrated that mRNAs for UGT1A5, UGT2B1 and UGT2B3 are also expressed in cultured liver cells. Moreover, the expression of UGT1A1 and UGT1A5 mRNAs but not UGT1A6 mRNA is increased by dexamethasone when added at a therapeutic concentration (13), which

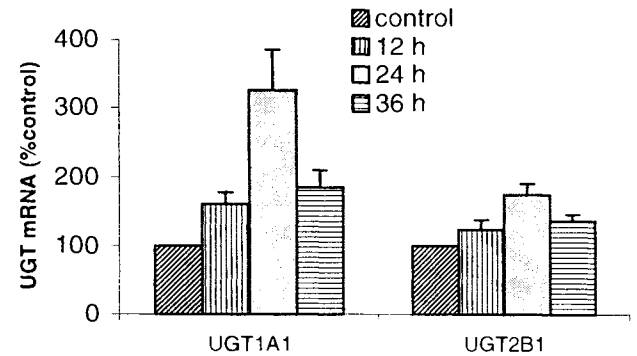

Fig. 4. Time course of UGT1A1 and UGT2B1 mRNA levels in cultured hepatocytes. Hepatocytes at day 4 were incubated with dexamethasone (DEX 10^{-6} M). Cells were harvested for RNA preparation at different time points. The mean of UGT mRNA concentrations normalized against 18s rRNA in two separate experiments are shown. Data are expressed as percentage of control values on the indicated times.

closely resembles their induction by dexamethasone *in vivo* (6). The mRNA levels of UGT2B1 and UGT2B3 were also enhanced by administration of dexamethasone but to a lesser degree compared with that of UGT1A1. To extend these observations, one isoform for each family (UGT1A1 and UGT2B1) was further examined in both of time-dependent and dose-dependent experiments. The induction of UGT1A1 and UGT2B1 mRNAs by dexamethasone were both dose- and time-dependent. These data correlating the results in hepatocyte culture to *in vivo* findings suggest that primary hepatocyte culture represents a suitable model for investigating hormonal effects on UGT gene expression and their mechanism.

Inhibition by cycloheximide of dexamethasone-induced gene expression has been reported for several hepatic genes, such as albumin, tryptophane 2,3-dioxygenase, and tyrosine aminotransferase gene (14–16). The present study showes that cycloheximide has a similar inhibitory effect on dexamethasone stimulated expression of UGT1A1 and UGT2B1 mRNAs, indicating that *de novo* protein synthesis is involved. In other words, these results suggest that dexamethasone induces the synthesis of a mediator protein which in turn up-regulates the expression of UGT. The increased mRNA levels of UGT following dexamethasone do not appear to be due to decreased rate of mRNA degradation since dexamethasone did not alter UGT mRNA stability when transcription was halted by exposure of hepatocytes to DRB. Thus it is probable that enhanced UGT gene transcription is responsible for the increased UGT activities and mRNA levels in hepatocytes treated by dexamethasone. Glucocorticoid activates transcription in a number of genes such as the metallothionein-I gene in the liver (17) and the GH gene in the pituitary (18). This is considered to result from association of the hormone-receptor complex with a specific DNA sequence, termed the glucocorticoid response element (19,20). However, a nuclear run-on transcription assay would be needed to demonstrate such increased transcription rate by dexamethasone applies in the case of the UGT.

Sex difference in the glucuronidation of a variety of compounds has been widely reported (3,21). Thus it is worth studying whether any sex hormone has a direct role in the regulation of UGT. Addition of testosterone resulted in about 30% increase in both UGT2B1 and UGT2B3 mRNAs. This result is in agreement with data previously obtained *in vivo* (4). However, the concentration of testosterone used in hepatocyte culture was superphysiological, suggesting that the effect of testosterone on the expression of UGT2B1 and UGT2B3 *in vivo* might be indirect. Nemoto and Sakurai reported that exposure of hepatocytes for 72 h to 10^{-5} M estradiol and progesterone induced the expression of cytochrome (CYP2b-9 and CYP2b-10) (22), but in this study these concentrations had no effect on either UGT 1 or UGT 2 family genes. This result is consistent with lack of effect *in vivo* of estradiol injection on the expression of UGT 1A1 and UGT 2B3 (7). Thus the sex difference in the glucuronidation of bilirubin, phenol and testosterone found *in vivo* cannot easily be explained by a direct role of female hormones on the liver (3,21).

The expression of some sex specific cytochrome P450 isoforms such as P4502C12 is found to be dependent on growth hormone (23). In this study, administration of growth hormone at a superphysiological concentration of 10 mU (8800 ng/ml) was associated with only slightly increased expression of

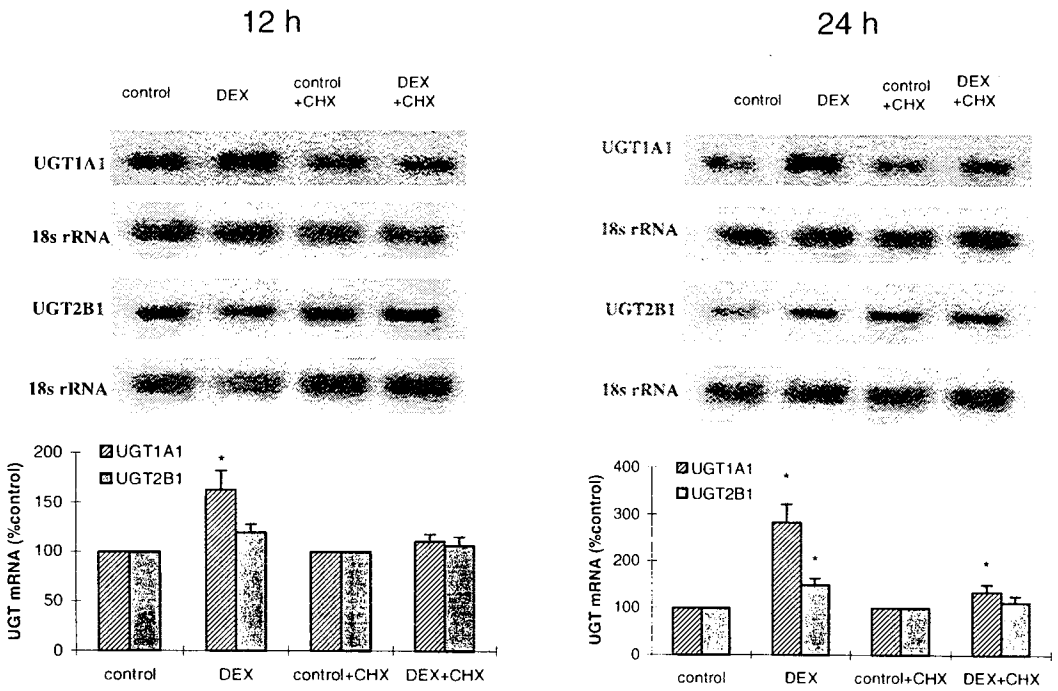

Fig. 5. Effect of cycloheximide on the induction of UGT 1A1 and UGT2B1 mRNAs by dexamethasone in cultured rat hepatocytes. Hepatocytes at day 4 were incubated with dexamethasone (DEX 10^{-6} M) and cycloheximide (CHX 10 μg/ml) simultaneously for 12 h and 24 h and cells were harvested for RNA preparation 12 h and 24 h later, respectively. The graph summarizes the UGT mRNA concentrations normalized against 18s rRNA in three separate experiments. The results are expressed as a percentage of the control, which is set at 100%. * $p < 0.05$.

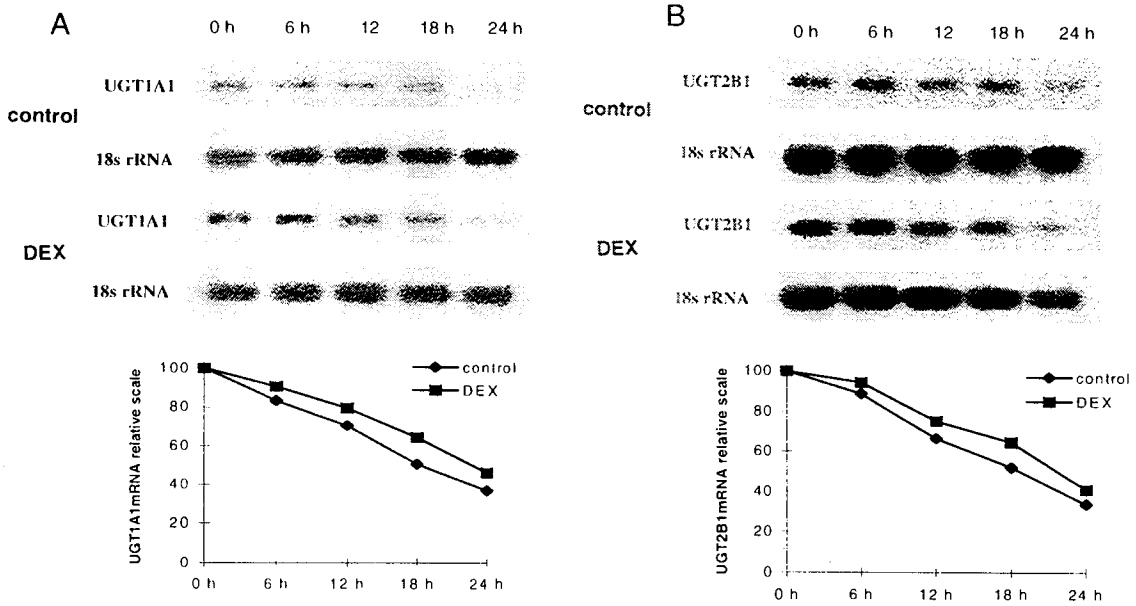

Fig. 6. Effect of 5,6-dichloro-1-D-ribofuranosylbenzimidazole (DRB) on the stability of UGT1A1(A) and UGT2B1(B) mRNAs induced by dexamethasone in cultured rat hepatocytes. Hepatocytes on day 4 were treated with dexamethasone (DEX 10^{-6} M) for 24 h. DRB (25 μg/ml) was then added to control and dexamethasone-treated hepatocytes. Cells were harvested for RNA preparation at 0, 6, 12, 18, 24 h after the addition of DRB. The graph summarizes the UGT mRNA concentrations normalized against 18s rRNA, each point represents the average value from two separate experiments.

UGT2B1 and UGT2B3 mRNAs compared with that of hepatocytes cultured without growth hormone. Even these levels did not significantly alter the mRNA levels of UGT 1family isoforms studied in this assay. The lack of effect of growth hormone on UGT 1A1 and UGT 1A5 mRNA levels agrees with recently published results *in vivo*, although UGT 1A2, another member of UGT1 family which we did not study, has been found to be significantly affected by growth hormone in rat liver (24).

The action of growth hormone on hepatocytes is affected by the timing of their exposure to the hormone. Thus hepatocytes from male rats express both mRNA and protein of female specific cytochrome CYP2C12 when exposed continuously to growth hormone, as was done in the experiments reported in this paper. If such continuous exposure similarly affects the expression of UGT, it might have been expected that the female pattern of expression would be seen in hepatocytes from male rats. *In vivo* male rats glucuronidate *p*-nitrophenol more rapidly and bilirubin more slowly than females (2,3). Therefore, change to the female pattern would entail a decrease in UGT1A6 and an increase in UGT1A1 and UGT1A5 that are responsible for glucuronidation of *p*-nitrophenol and bilirubin respectively. The absence of such a change argues against growth hormone mediating the sex difference in these glucuronidation pathways.

Our study demonstrates that addition of T3 to the cultured hepatocytes increases the expression of UGT 1A1 and UGT 1A5 mRNAs, but decreases the expression of UGT 1A6 mRNA. These results agree with the *in vivo* data reported by Masmoudi *et al.* (5). The molecular mechanism by which T3 treatment results in opposite effects on mRNAs for UGT1A1 and UGT1A5 and UGT1A6 is unknown. UGT1A1, UGT1A5 and UGT1A6 are all made from the UGT family 1 gene complex by alternatively splicing unique exon 1 with commonly used exons 2–5 (6). It is probable that the different regulation of T3 on UGT family 1 isoforms results from the diversity of promoter elements in the individual genes because each gene in UGT 1 family has its own promoter (25). The interaction of the nuclear thyroid hormone receptor (TR) with other transcription factors in the signaling pathways of TR may also be involved. The diverse effects of T3 in the gene regulation have been found to require cooperation between TR and a large number of transcription factors termed thyroid hormone receptor-associated proteins including co-activators or co-suppressors (26). Recently, Masmoudi *et al.* reported that UGT1A6 gene was regulated by T3 at transcription level and ongoing protein synthesis was required for the full induction (27). Identification and characterization of mediator proteins that regulate the transcriptions of UGT1A6 should be helpful in understanding the relationship between TR and other transcription factors in the permissive action of T3 on UGT gene expression.

T4 is the major product secreted by the normal thyroid gland in both humans and rats, but its intrinsic bioactivity is low. However, Goudonnet *et al.* reported that *in vivo* T4 produced opposite effects on glucuronidation of 4-nitrophenol and bilirubin similar to those of T3, when injected in the rats (28). Thus we evaluated whether T4 has a direct role in the expression of UGT mRNA by using cultured hepatocytes. The results did not show any effect of T4 on the mRNA levels of five UGT isoforms studied in this assay, suggesting that T4 regulates UGT enzyme activities indirectly, probably through conversion to T3 *in vivo*.

In summary, we have demonstrated that multiple hormones take part in the regulation of UGT mRNA expression in the rat and individual genes can be differentially modulated. The regulation of UGT mRNAs by dexamethasone may occur at transcription level and requires *de novo* protein synthesis.

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