# Sex Hormones Differentially Regulate Isoforms of UDP-Glucuronosyltransferase

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***Purpose.*** To investigate the role of sex hormones in the regulation of UDP-glucuronosyltransferase (UGT).
***Methods.*** We examined liver from adult, prepubertal, gonadectomised and gonadectomised plus hormone replaced rats of both sexes. Immunohistochemistry and immunoblots were performed using a polyclonal UGT antibody to a number of family 1 and family 2 UGT isoforms. Northern blot analysis was performed utilising cDNA probes to family 1 and family 2 isoforms.
***Results.*** Immunohistochemistry demonstrated variations in intensity and distribution of staining in the hormonally manipulated rats. Immunoblots showed variations in individual band intensity between rat groups. Immunoblots using a more specific antibody (anti-17β-hydroxysteroid UGT, which recognises UGT2B3 and UGT2B2) demonstrated marked differences between male and female rats and significant alterations after gonadectomy and testosterone replacement in the male rats. In northern analysis, UGT2B3 and 2B1 mRNA were significantly higher in adult males than females, and in prepubertal males compared to prepubertal females. In male rats, gonadectomy resulted in a 45–53% reduction in UGT2B3 and 2B1 levels respectively, which increased significantly with testosterone treatment to greater than normal adult levels. No change in UGT2B3 or 2B1 occurred after gonadectomy in females. In contrast, UGT1*1 mRNA tended to be higher in adult female and prepubertal female rats than in their male counterparts. In females, gonadectomy resulted in significant up-regulation of UGT1*1, while gonadectomy plus oestradiol treatment resulted in markedly reduced levels. UGT1*1 mRNA was not significantly altered by gonadectomy in males.
***Conclusions.*** This study demonstrates the differential effects of sex hormones on the expression of isoforms from the two phylogenetically distinct UGT families.

**KEY WORDS:** drug metabolising enzyme; oestradiol; testosterone; UDP-glucuronosyltransferase.

## INTRODUCTION

The UDP-glucuronosyltransferases (UGT) are a family of membrane-bound enzymes responsible for the glucuronidation of many endogenous and exogenous compounds. In general, glucuronidation is a detoxifying process, whereby a lipophilic compound is rendered water soluble and more easily excreted from the body. However importantly, glucuronidation can also result in the production of active and toxic metabolites (1,2). The activity of UGT is altered by many factors including specific enzyme inducers, aging, diet and disease states (3–5). In addition, clear sex differences in UGT activity exist (6). In these situations, regulation may occur at the level of gene transcription resulting in changes in mRNA and protein levels, at the level of post-translational processing, particularly glycosylation, or may be due to changes to the functional state of the enzyme by modification of its membrane environment. Availability of co-substrates is also an important determinant of activity (7).

In recent years the existence of a UGT gene superfamily has been clearly demonstrated, accounting for the observed variability in substrate handling and ontogeny. In rats, at least twelve isoforms have been cloned and classified into two distinct families based on amino acid sequence homology (8). Family 1 isoforms (*UGT1*), derived by alternate splicing of the primary transcript of a single large gene, are responsible for glucuronidation of a range of substances including bilirubin small planar phenols and also some steroids and drugs (4). Family 2 isoforms (*UGT2*) are encoded by separate genes located on a different chromosome and glucuronidate a range of unrelated steroids, bile acids, drugs and also some phenols (4). Once an isoform has been cloned, its nucleotide sequence and cDNA can be used in the study of the regulation of UGT gene expression.

Sex hormone regulation of UGT enzyme activity towards a variety of substrates has been demonstrated (9–11). However, while suggesting differential regulation of UGT isoforms, studies to date have only described sex differences or sex hormone modification of the activity of the enzyme to individual substrates. Substrates used in these studies can usually be glucuronidated by more than one UGT isoform, not allowing for assessment of the regulation of individual isoforms. No studies have addressed sex hormone regulation of UGT at a molecular level and thus the relative contributions of changes in protein synthesis or alterations in the functional state of the enzyme protein, have not been clearly defined.

The aim of this study was to explore the regulation of UGT by sex hormones, by examining their effects on UGT protein concentrations and on the steady state mRNA levels of individual UGT isoforms from the two gene families.

## MATERIALS AND METHODS

### Animals and Treatments

Sprague-Dawley rats (Biological Research Laboratories, Austin Hospital, Melbourne) were housed using a 12 hour light cycle and allowed unrestricted access to food and water. The study was approved by St. Vincent's Hospital animal ethics committee and the research adhered to the "NIH Principles of Laboratory Animal Care."

The following groups of rats were studied: (i) adult males and females (12 weeks of age); (ii) prepubertal males and females (5 weeks of age); (iii) gonadectomised (or sham operated) males and females; and (iv) gonadectomised males and females treated with testosterone and oestradiol respectively. At least three rats per group were studied. For the experiments in gonadectomised rats, a protocol similar to that of Muraca and Fevery (10) was followed. Surgery was performed on 7 week old rats under pentobarbitone anaesthesia (50 mg/kg body

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weight) (Nembutal, Boehringer Ingelheim, Artarmon, Australia). In males, gonadectomy was performed via a midline scrotal incision, and in females through a midline dorsal incision. In order to study the effect of sex hormone replacement, males received a slow release preparation of testosterone enanthate, 25 mg, (Primoteston depot, Schering P/L) (or vehicle) by subcutaneous injection on the day of operation and ten days later. A slow release preparation of oestradiol valerate, 1 mg, (Primogyn depot, Schering P/L) (or vehicle) was given to females on the day of operation and at ten days. Sham operated animals underwent anaesthesia and relevant incisions. The animals were studied twenty-one days after operation. All animals were killed by decapitation and their livers rapidly excised, snap frozen in liquid nitrogen and stored at −70°C. These experiments were approved by the St Vincent's Hospital Animal Experimentation Ethics Committee.

### Serum Hormone Levels

Blood for serum hormone levels was collected at the time of decapitation from 4–11 rats per group. Serum was stored at −70°C and thawed only once. Serum testosterone was assayed by enzyme linked fluorescent assay (bioMerieux, Lyon, France) and oestradiol by radioimmunoassay (Diagnostic Products Corporation, Los Angeles, USA).

### Immunohistochemistry

The expression of UGT in liver sections from three animals per group was assessed by immunohistochemistry as previously described (12). A polyclonal anti-UGT antibody recognising multiple *UGT2* family isoforms and at least one *UGT1* family isoform was used in immunostaining for UGT. The characteristics of this antibody are described below.

### Preparation of Hepatic Microsomes and Immunoblotting

Microsomes were prepared from rat liver as described previously (12) and were stored in sucrose/EDTA at −70°C until use. Protein content was assayed by the method of Lowry (13).

Microsomal protein (5 μg) was resolved by SDS-polyacrylamide gel electrophoresis and transferred to nitrocellulose filters. UGT protein was detected by immunoblotting as previously described (12).

### Anti-UGT Antibodies

The antibodies used in this study were:

(i) a polyclonal anti-UGT antibody; raised in goats to purified mouse UGT. The antibody has been fully characterised and recognises multiple rat UGT isoforms, including UGT2B1, UGT2B2, UGT2B3 and isoforms from family 1 (14–17). The antibody was kindly supplied by Dr P. I. Mackenzie, Bedford Park, South Australia.

(ii) anti-17β-hydroxysteroid (testosterone) UGT; a polyclonal antibody raised in female New Zealand white rabbits (18). The rat hepatic microsomal 17β-hydroxysteroid UGT monomer used to produce this antibody, was isolated and purified by chromatofocusing and affinity chromatography, and the monomeric subunit isolated by means of preparative SDS-polyacrylamide gel electrophoresis (19). The antibody has been fully characterised (18). In immunoblotting, the antibody reacts with purified 17β-hydroxysteroid UGT (50 kDa; UGT2B3) as well as purified 3α-hydroxysteroid UGT (52 kDa; UGT2B2). It also recognises a third UGT protein band, of molecular weight greater than 52 kDa). It does not recognise family 1 UGTs. The anti-17β-hydroxysteroid UGT antibody was kindly supplied by Dr. T.R. Tephly, Iowa, USA.

#### *Complementary DNA Probes*

The nomenclature of UGT isoforms used here follows the recommended system (8). Full length cDNA probes to UGT2B3 (20) and UGT2B1 (21) were supplied by Dr Peter Mackenzie of Flinders Medical Center, Bedford Park, South Australia). A 913 base pair cDNA to exon 1 of UGT1*1 (22) was kindly supplied by Dr Takashi Iyanagi of Himeji Institute of Technology, Akogun Hyogo, Japan.

#### *Total RNA Extraction and Northern Hybridisation*

Total RNA was prepared from liver by the method of Chomczynski and Sacchi (23) and then stored in 0.5% SDS at −70°C. RNA (20 μg) was subjected to denaturing agarose gel electrophoresis (1.5% agarose), transferred to Hybond-N nylon filters (Amersham, Buckinghamshire, U.K.) and fixed by UV irradiation. Filters were hybridised overnight with $\alpha$-$^{32}$P-labelled cDNA probes prepared by random priming (Dupont, Sydney, Australia). Washes consisted of 2xSSPE/0.1%SDS at 42°C for 15 minutes, 1xSSPE/0.1%SDS at 37°C for 30 minutes and 0.1xSSPE/0.1%SDS at 20°C for 15 minutes. Quantitation of mRNA levels was by scanning densitometry of autoradiographs. To assess and correct for loading of gels, filters were reprobed with an oligoprobe to the 18S ribosomal band. Specific UGT band densities were then expressed as a ratio to the density of the 18S band.

### Statistical Analysis

The levels of UGT mRNA determined by scanning densitometry are shown in graphical form as the mean ± standard error of the densitometric value normalised to the corresponding 18S ribosomal band intensity. Differences between groups were determined by ANOVA and were considered significant if $p < 0.05$.

## RESULTS

### Serum Hormone Levels

The mean (±SEM) serum testosterone level in pre-pubertal male rats was 0.8 ± 0.6 ng/dL and in adult males, 3.1 ± 0.9 ng/dL. Following gonadectomy, testosterone was undetectable in serum. After dosing with subcutaneous testosterone enanthate, the mean level was 24.8 ± 7.9 ng/dL.

The mean (±SEM) serum oestradiol level in pre-pubertal female rats was 39.3 ± 28.6 pg/mL. In adult females the mean level was 160.0 ± 56.4 pg/mL. Following gonadectomy the level fell to 67.9 ± 27.3 pg/mL. After subcutaneous oestradiol, very high levels of oestradiol (10160 ± 2328 pg/mL) were detected.

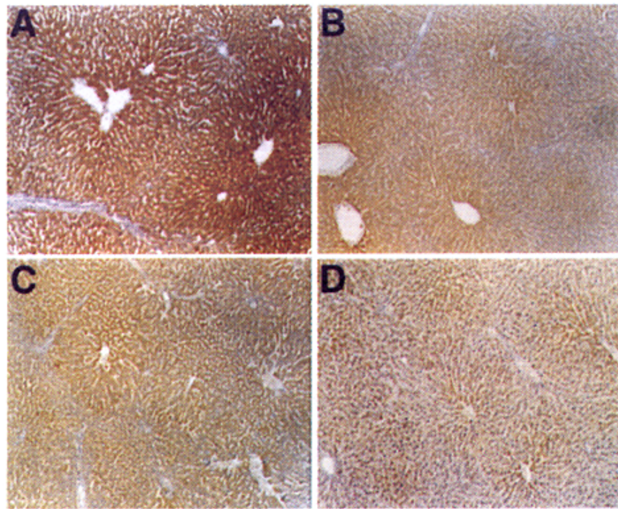


**Fig. 1.** Immunohistochemical staining for UGT using a polyclonal antibody in (A) adult male (B) adult female (C) pre-pubertal male and (D) pre-pubertal female Sprague-Dawley rats. The staining is predominantly pericentral and the staining in adult males is greater than in the other three groups.

## Immunohistochemistry

Immunohistochemistry was performed using the polyclonal UGT antibody against both family 1 and family 2 isoforms. Photomicrographs of UGT immunostaining in liver sections from representative rats are shown in Figures 1 and 2.

### *Untreated Rats*

While UGT immunoreactivity was present in all hepatocytes, the level of expression was not homogeneous with most intense immunostaining in hepatocytes around the central vein of the hepatic lobule (pericentral region). The degree of staining was greater in liver from adult male rats (Figure 1A) compared to that from prepubertal male (Figure 1C) and female (Figure 1B) or adult female rats (Figure 1D).

### *Hormone Manipulated Rats*

Immunohistochemical staining for UGT in livers from gonadectomised male rats was again most intense in pericentral hepatocytes (Figure 2A). However, compared to sham operated males (Figure 2C), the staining appeared more contracted around the central vein, with some reduction in staining in periportal and midzonal hepatocytes. In rats treated with testosterone after gonadectomy, UGT immunostaining increased markedly throughout the hepatic lobule (Figure 2B).

Immunostaining for UGT in livers from female rats was of lower intensity than in males, and more evenly distributed throughout the hepatic lobule (Fig. 2F). Gonadectomy alone (Figure 2D) or with oestradiol replacement (Figure 2E) did not significantly affect the intensity or distribution of staining.

## Immunoblotting

### *Polyclonal Anti-UGT Antibody*

#### *Untreated Rats*

Immunoblot analysis using the polyclonal anti-UGT antibody, revealed at least four distinct immunoreactive bands in hepatic microsomes from all groups of rats. However between groups of rats, the pattern of bands varied. A very broad lower band was present at approximately 50 kDa, in microsomes from prepubertal females, prepubertal males and adult females which likely represented immunoreactivity to more than one UGT isoform.

The pattern in adult male rats differed, with a more distinct band at 50 kDa possibly corresponding to a single isoform. In contrast to the other groups studied, male rats also exhibited an equally distinct higher molecular weight band at approximately 52 kDa. In other groups, this band was less prominent.

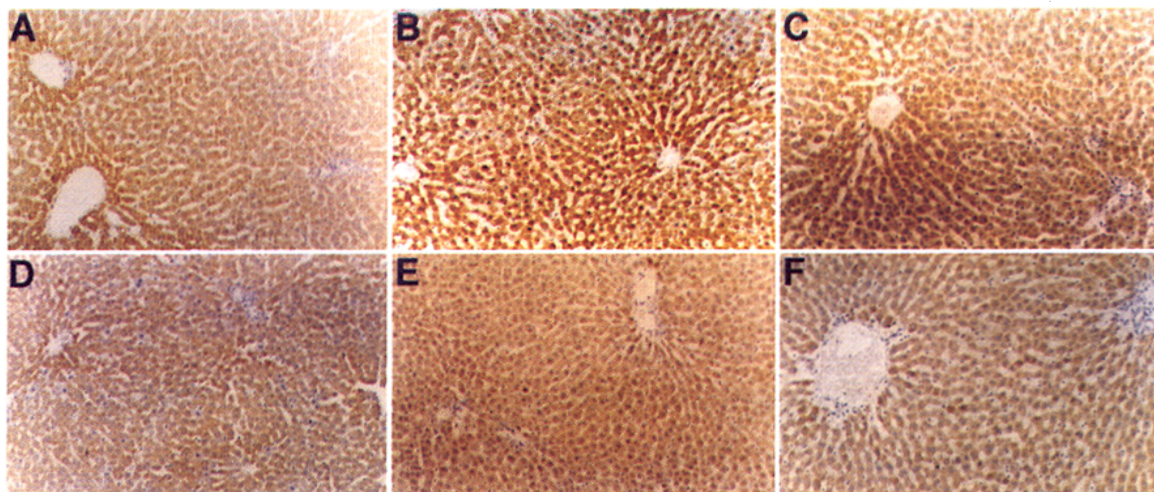


**Fig. 2.** The effects of hormone manipulation on immunohistochemical staining for UGT in rat liver. (A) gonadectomised male; (B) gonadectomised male treated with testosterone enanthate; (C) sham operated male; (D) gonadectomised female; (E) gonadectomised female treated with oestradiol valerate; (F) sham operated female. In males, staining was reduced after gonadectomy and markedly increased after testosterone treatment In contrast, in females gonadectomy without or with oestradiol replacement did not significantly affect the intensity or distribution of staining.

In all rats, higher molecular weight species were also detected by immunoblotting, although no clear differences between groups could be seen.

*Hormone Manipulated Rats*

In adult males (sham operated), two major immunoreactive UGT bands were present, corresponding to the 50 and 52 kDa bands described above. No clear difference was seen following gonadectomy. With testosterone replacement after gonadectomy (to supraphysiological levels), the lower molecular weight band became broader, consistent with the presence of increased amounts of one or more UGT isoforms. In contrast, with testosterone treatment, the upper, higher molecular weight band became less prominent compared to sham operated or gonadectomised rats.

In the gonadectomy study in female rats, interindividual variability of UGT immunoreactivity was seen to a much greater degree than in the males.

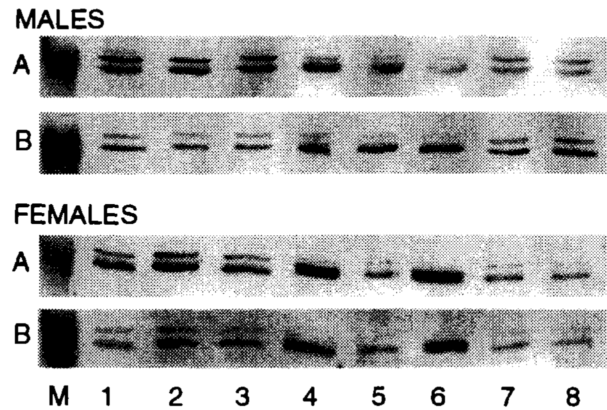


**Fig. 3.** Immunoblot analysis of UGT protein in hepatic microsomes from rats after gonadectomy (Lanes 1–3), gonadectomy plus treatment with either testosterone (males) or oestradiol (females) (Lanes 4–6), and in sham operated rats (Lanes 7–8). A: polyclonal anti-UGT antibody and B: anti-17β hydroxysteroid UGT antibody. Lane M: 49.5 kDa molecular weight marker.

*Anti-17β-Hydroxysteroid (Testosterone) UGT*

*Untreated Rats*

In hepatic microsomes from prepubertal female rats, prepubertal males and adult females, immunoblot analysis revealed one dominant immunoreactive band, corresponding to the 50 kDa subunit, 17β-hydroxysteroid UGT (UGT2B3), as described by Knapp *et al.* (18). No difference was seen in the intensity of staining in these three groups of animals, suggesting equivalent UGT protein levels per gram of microsomal protein. In males rats however, two immunoreactive bands were present, corresponding to 17β-hydroxysteroid UGT (50kDa; UGT2B3) and 3α-hydroxysteroid UGT (52 kDa; UGT2B2) (18). The intensity of the lower molecular weight band was greater than the higher molecular weight band, and was greater than the corresponding band in the other three groups of rats.

*Hormone Manipulated Rats*

Use of the more specific anti-17β-hydroxysteroid UGT antibody, permitted clarification of the changes in patterns of immunoreactivity noted with the polyclonal antibody. In male rats, the lower molecular weight band (17β-hydroxysteroid UGT, UGT2B3) decreased in intensity following gonadectomy and increased following testosterone treatment to greater than the level in sham operated males. In contrast, the higher molecular weight band (3α-hydroxysteroid UGT, UGT2B2), was little changed following gonadectomy, but reduced in intensity after testosterone treatment. These results suggested that the broad lower molecular weight band seen with the polyclonal anti-UGT antibody indeed comprised a number of isoforms that were differentially regulated in this model.

In female rats undergoing gonadectomy, a clearer pattern also emerged when the 17β-hydroxysteroid UGT antibody was used in immunoblot analysis. With this antibody, two major bands were again present. Gonadectomy did not alter the intensity of the lower molecular weight band, however the higher molecular weight band became more prominent. Oestradiol treatment resulted in broadening of the lower molecular weight band in two of three rats studied, suggesting possible induction of another UGT isoform.

Representative immunoblots are shown in Figure 3.

**Northern Blot Analysis**

*UGT2B3 Probe*

The UGT2B3 mRNA levels in each group of rats studied are shown in Figure 4 (A–C). The level of UGT2B3 mRNA was significantly higher in males than in females. This difference was seen in adult animals (males 170% of females $p < 0.05$)) and in prepubertal rats (males 185% of females, $p < 0.05$) Gonadectomy in adult male rats led to significant down-regulation of its expression to 55% of the level found in sham operated animals ($p < 0.05$). Hormone replacement therapy to restore the circulating testosterone levels to greater than that seen in controls, was associated with a marked up-regulation of expression. Gonadectomy in females, with or without oestradiol treatment, resulted in little change in UGT2B3 mRNA levels.

*UGT2B1 Probe*

The results of studies of UGT2B1 mRNA levels in these groups of rats were similar to those for UGT2B3. Levels of UGT2B1 mRNA were significantly higher in adult and prepubertal males rats, compared to both groups of female rats ($p < 0.05$). Gonadectomy in adult males resulted in a 53% reduction in the level of UGT2B1 mRNA ($p < 0.05$). Testosterone replacement after gonadectomy led to a 236% increase in levels compared to rats undergoing gonadectomy alone, a level of 110% of the that seen in sham operated adult males. As with UGT2B3, no significant differences were seen in hormone-manipulated females.

*UGT1*1 Probe*

The *UGT1* isoform, UGT1*1, responded quite differently to hormonal manipulation (Figure 4, D–F). While not attaining statistical significance, the mean hepatic mRNA level of

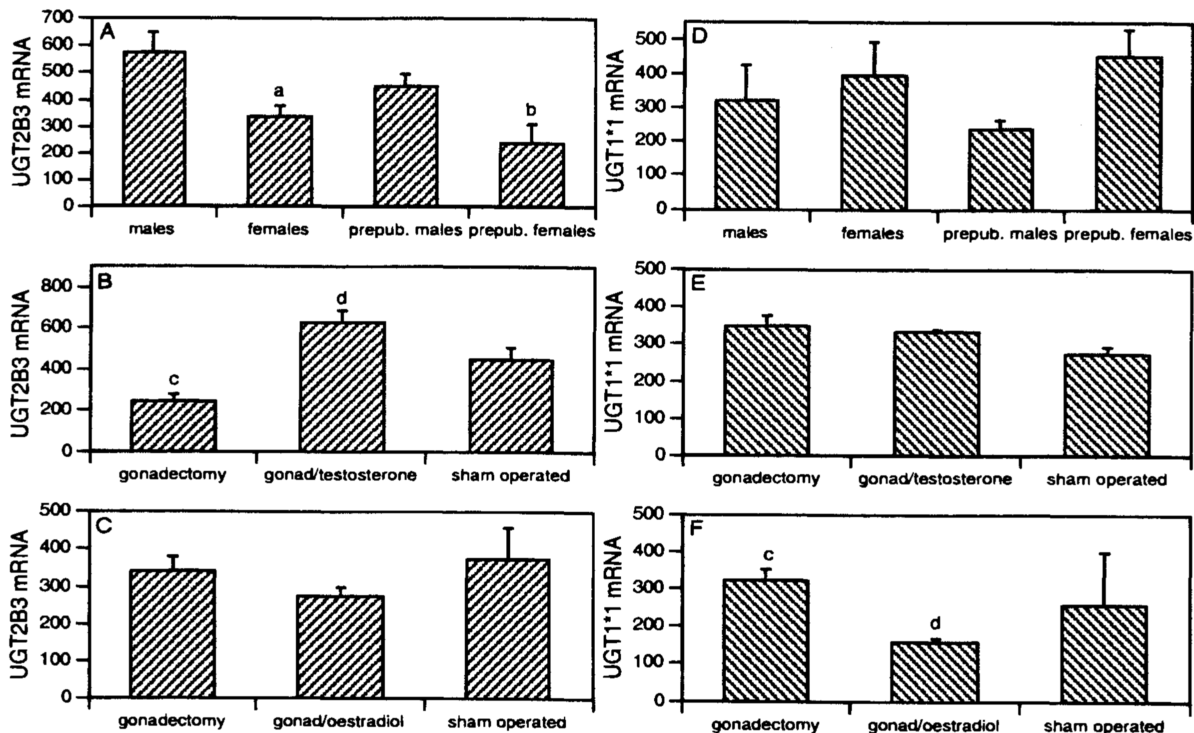


**Fig. 4.** Northern blot analysis for UGT2B3 (A-C) and UGT1*1 (D-F) in liver of Sprague-Dawley rats. (A) and (D): adult male, adult female, prepubertal male and prepubertal female; (B) and (E): male rats after gonadectomy, after gonadectomy and testosterone treatment and with sham operation; (C) and (F) female rats: after gonadectomy, after gonadectomy and oestradiol treatment and after sham operation. Results expressed as mean ± SEM from three animals (arbitrary units).
[a]$p < 0.05$ compared to males; [b]$p < 0.05$ compared to prepubertal males; [c]$p < 0.05$ compared to shams; [d]$p < 0.01$ compared to gonadectomy.

UGT1*1 was higher in adult female and prepubertal female rats than in their male counterparts. Gonadectomy in females resulted in significant up-regulation of UGT1*1 (by 26%) compared to the level in sham operated animals ($p < 0.05$). Treatment with oestradiol resulted in marked down-regulation below the level in shams. In males, gonadectomy with or without testosterone treatment resulted in little change in the level of UGT1*1 mRNA.

## DISCUSSION

This study provides evidence that altered protein synthesis may in part explain the previously observed sex differences and hormonal regulation of UGT activity. UGT protein content and mRNA levels for different isoforms vary between male and female rats, between adult and prepubertal animals, and in their response to hormonal manipulation. This suggests that sex hormones play a role in regulating the expression of *UGT1* and *UGT2* family isoforms.

Sex-hormone dependent differences in UGT expression were detectable by immunohistochemical staining of liver sections. The expression of UGT was highest in adult males, compared to females and prepubertal animals. In male rats, the reduction in intensity of UGT immunostaining following gonadectomy, and the markedly increased staining after administration of exogenous testosterone, suggested that levels of circulating testosterone might regulate UGT (and in particular, *UGT2*) expression.

While immunohistochemistry is a useful tool for studying major changes in the distribution and abundance of proteins, the interpretation of results is determined by the specificity of the antibody used. As the anti-UGT antibody used in this study was not isoform-specific, selective regulation of isoforms might have been masked. For instance if a manipulation results in down-regulation of one isoform while another is up-regulated, the overall intensity of immunostaining may not alter.

The results of immunoblot analysis however, extended the immunohistochemical findings and demonstrated the effects of sex hormones, in particular testosterone, upon individual *UGT2* family isoforms. The results supported a role for testosterone in regulating the expression of 17β-hydroxysteroid UGT (encoded by UGT2B3). This isozyme was present in highest levels in hepatic microsomes from adult male rats, was reduced following orchidectomy and increased with testosterone treatment. In addition, the results suggested that circulating testosterone levels might also regulate expression of 3α-hydroxysteroid UGT (UGT2B2).

Northern blot analyses of isoform-specific mRNA levels, lent further support to the findings of sex hormone regulation of UGT expression. The study demonstrates isoform dependent differences of steady state UGT mRNA levels between male and female rats and between adult and prepubertal animals. In addition, we have demonstrated isoform dependent changes in response to manipulation of the hormonal environment. These results suggest that sex hormones play a role in regulating

*UGT1* and *UGT2* isoforms at a pre-translational level and are responsible for the observed changes in UGT protein content.

Our results suggest that testosterone is an important regulator of some *UGT2* isoforms rather than *UGT1* isoforms. We have demonstrated higher levels of UGT2B1 and UGT2B3 mRNA in males compared to females and following testosterone treatment of castrated males. In addition, gonadectomy resulted in significant down-regulation of these isoforms. It is interesting to speculate as to the physiological significance of this finding as the main substrates for these *UGT2* isoforms are endogenous steroids, particularly androgens (20,21). However, the results relating to regulation of *UGT2* isoforms by oestrogens were inconclusive. While differences in protein content were demonstrated in females following gonadectomy and with hormone replacement, the changes did not correspond to specific regulation of UGT2B1 or 2B3, suggesting regulation of another family 2 isoform, possibly UGT2B2.

In comparison to the findings with family 2 isoforms, testosterone had little apparent role in regulating UGT1*1. The genes encoding *UGT1* isoforms differ from those encoding *UGT2* isoforms in that they are all derived from a single large gene with individual transcripts generated by alternate splicing (8). Substrates for these isoforms are generally phenols or bilirubin, rather than steroids. In this study UGT1*1, which glucuronidates a range of compounds including bilirubin, 1-naphthol and 7,7,7-triphenylheptanoic acid (24,25), was more sensitive to the effect of gonadectomy in females, with a significant rise in mRNA levels which were suppressed by high levels of circulating oestradiol, suggesting a possible role for oestrogen in its regulation.

Other factors must also be important to explain the changes of UGT mRNA levels demonstrated in this study. For instance, UGT2B1 and UGT2B3 mRNA levels were higher in prepubertal male than prepubertal female rats. These findings contrasted with those of immunoblotting, in which no difference in UGT protein content was present in prepubertal males compared to adult or prepubertal females. Study of larger numbers of animals may resolve this discrepancy. However if confirmed, the results suggest that factors other than circulating testosterone levels may be important, for instance levels and pattern of secretion of pituitary hormones such as gonadotropins and growth hormone. Certainly some cytochrome P450 isoforms are regulated at a transcriptional level by the sexually differentiated secretory pattern of growth hormone whereby males exhibit a highly pulsatile pattern compared to a nonpulsatile pattern in females (26,27). A similar mechanism has not been demonstrated for phase II enzymes.

Previous studies demonstrating alterations in UGT enzyme activity to a variety of substrates in the setting of changes in circulating sex steroids support our findings of differential regulation. Activity of UGT to the family 1 substrate *p*-nitrophenol, has been reported to be higher in male rats than in females (10,28,29) while activity towards another *UGT1* substrate, bilirubin, had higher activity in females (10). Glucuronidation of a variety of steroid hormones, metabolised by *UGT2* isoforms, has been demonstrated to be higher in female than male rats (9), although the effects of gonadectomy have not been studied.

Gonadectomy and subsequent hormone replacement have been shown to affect activity towards *UGT1* substrates differentially depending on the substrate studied. With phenolsulfonphthalein (11) or *p*-nitrophenol (30) as substrates, orchidectomy resulted in reduced activity which was restored with testosterone treatment. The opposite affect on phenolsulfonphthalein glucuronidation was seen with ovariectomy (11). These findings contrasted to the effects on bilirubin glucuronidation which reversibly increased after orchidectomy and decreased following ovariectomy (10).

These previous studies do not address the molecular basis of UGT regulation, but in many the results suggest alterations in protein synthesis. Certainly, hormonal treatment has been demonstrated to alter the phospholipid composition and fluidity of microsomal membranes (31). In theory, this may result in altered enzyme activity by changing the three dimensional structure and molecular dynamics of this membrane-bound enzyme (32). However, in a study of the influence of sex and sex steroids on bilirubin UGT activity (10) treatment of microsomes with detergent resulted in identical activation curves to untreated microsomes making it unlikely that changes in the membrane environment were responsible for regulation. Likewise, induction by testosterone of *p*-nitrophenol UGT activity has been demonstrated in detergent-activated microsomes from castrated male rats (30). Thus, from these studies it seemed likely that sex hormones, and in particular testosterone, exert their effects on UGT activity, at least in part, by altered enzyme synthesis.

In conclusion, this study has clearly demonstrated that sex hormones play an important role in the regulation of individual isoforms of UGT and this occurs at a pre-translational level.

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