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Characterization of UDP-glucuronosyltransferases active on steroid hormones ☆

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Abstract

In recent years, the enzymes which are involved in the formation of DHT in steroid target tissues have been well investigated, however, enzymes responsible for the catabolism and elimination of steroids in these tissues, in particular the uridine diphospho-glucuronosyltransferase (UGT) family of enzymes, have received much less attention. We have recently demonstrated that human and monkey are unique in having high plasma levels of C19 steroid glucuronides. These circulating conjugates have been proposed to reflect the peripheral conversion of adrenal and gonadal C19 steroids to potent androgens, especially DHT. In humans, the presence of steroid UGT activities is found in the liver and several extrahepatic tissues including the prostate, mammary gland and ovary. In addition, UGT activities were observed in breast and prostate tumor cell lines such as MCF-7 and LNCaP, respectively. In agreement with the presence of steroid conjugating enzymes in extrahepatic tissues, UGT cDNA clones, which encode steroid conjugating proteins, have been isolated from libraries constructed from human and monkey prostate mRNA. The presence of UGT transcripts and proteins in extrahepatic tissues in both species, as determined by Northern blot, ribonuclease protection, specific RT–PCR, in situ hybridization, Western blot and immunocytochemistry analysis, indicate the relevance of steroid glucuronidation in tissues other than the liver. Knowing that both the human prostate and the human prostate cancer LNCaP cell line express steroid metabolizing proteins, including UGT enzymes, regulation of UGT mRNA and protein levels, as well as promoter activity was studied in these cells. The results demonstrate a differential regulation between the two highly related isoforms UGT2B15 and UGT2B17, where only the expression of UGT2B17 was affected following treatments of LNCaP cells with androgens, growth factors or cytokines. Steroid conjugation by UGT enzymes is potentially involved in hormone inactivation in steroid target tissues, thus modifications in UGT expression levels may influence hormonal responses.

1. Introduction

Conjugation of compounds by glucuronidation is a pathway found in all vertebrates studied to date [1]. Uridine diphospho-glucuronosyltransferase (UGT) enzymes catalyze the transfer of the glucuronyl group from uridine 5′-diphosphoglucuronic acid to active endogenous and exogenous molecules having functional groups of oxygen, nitrogen and sulfur. The resulting glucuronide products are more polar, generally water soluble, less toxic and more easily excreted than the substrate molecule [1,2]. Examples of endogenous substrates that are glucuronidated include bilirubin, bile acids and steroids whereas, xenobiotics such as drugs and pollutants are also detoxified by UGT enzymes [2–6].

More than 35 different UDP-glucuronosyltransferase cDNA clones from six mammalian species, which

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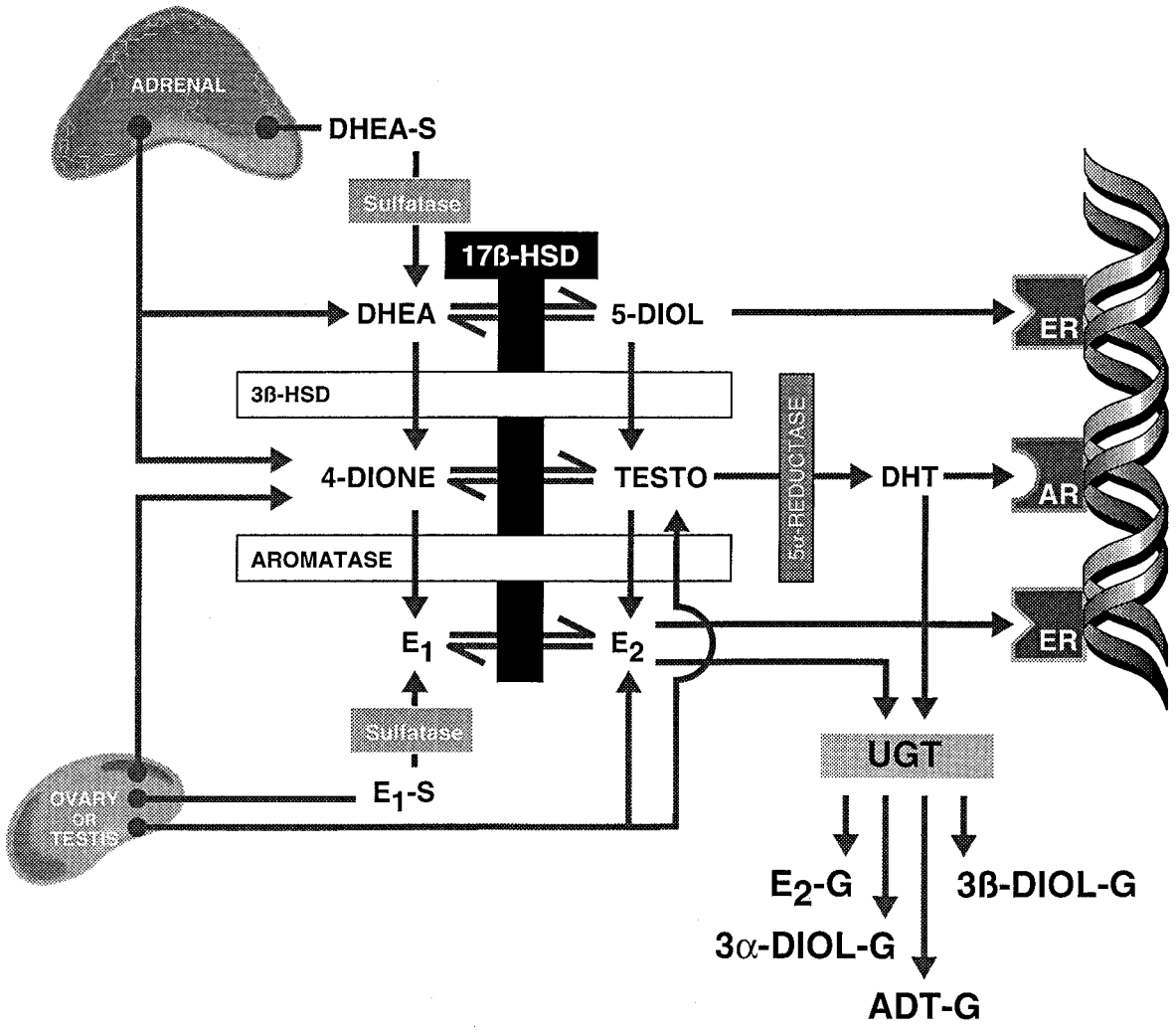

Fig. 1. Production and elimination of dihydrotestosterone (DHT) in steroid target cells. The concentration of DHT in the cell is in an equilibrium between production and elimination. The enzymes involved in steroid synthesis and catabolism are as indicated: UGT, uridine diphosphoglucuronosyltransferase; 3β-HSD, 3β-hydroxysteroid dehydrogenase; 17β-HSD, 17β-hydroxysteroid dehydrogenase; ADT, androsterone; 5-diol, androst-5-ene-3β,17β-diol; 3α-diol, androstane-3α,17β-diol; DHT, dihydrotestosterone; Testo, testosterone; 4-DIONE, androstenedione; DHEA, dehydroepiandrosterone; DHEA-S, dehydroepiandrosterone-sulfate; E_1, estrone; E_2, estradiol; E_1-S, estrone-sulfate; AR, androgen receptor; ER, estrogen receptor; DHT-G, DHT-glucuronide; 3α-diol-G, 3α-diol-glucuronide; ADT-G, ADT-glucuronide; 3β-diol-G, 3β-diol-glucuronide.

include 18 human UGT clones, have been isolated and characterized. Based on the homology of UGT primary structures deduced from nucleotide sequences, the mammalian UGT proteins have been categorized into two major families, UGT1 and UGT2 [6,7]. In humans, the *UGT1A* gene family is located on chromosome 2q37, where each *UGT1* gene is composed of a unique first exon and four common exons 2 to 5 [8]. To date, there has been 12 first exons of the UGT1 gene family which has been described in humans [8] and the rat [9]. The physiological importance of UGT enzymes is demonstrated by mutations in *UGT1A* genes, which cause several diseases with varying degrees of hyperbilirubinemia (Crigler-Najjar Type-I and II, and Gilbert's disease) [8,10–14]. These mutant genes demonstrate that decreased or absent levels of some UGT activities can lead to toxic levels of substrate accumulation in the body. It has been thought that substrates for UGT1A enzymes are restricted to bilirubin and xenobiotics; however, several recent reports demonstrate that UGT1 can also glucuronidate steroids [15–20].

Enzymes of the UGT2 family are divided into the three subfamilies UGT2A, UGT2B and UGT2C. To date, two UGT2A cDNAs have been described. UGT2A1 in both the rat and bovine is expressed in the olfactory epithelium involved in odorant sensory perception. It has been proposed that the mechanism

of sensory perception utilizes glucuronidation as a means to rapidly inactivate an odorant molecule, thus indicating that UGT enzymes provide an effective mechanism of signal termination [21]. In the absence of glucuronidation, activation of sensory perception by the odorant molecule persists for a longer period of time.

UGT2B enzymes on the other hand catalyze the glucuronidation of bile acids, steroids, fatty acids, carboxylic acids, phenols and carcinogens [7]. Six human UGT2B cDNA clones which encode steroid conjugating enzymes have been characterized. It is apparent that the UGT2B enzymes characterized thus far can glucuronidate xenobiotics such as eugenol and 4MU, however, their conjugation of steroid substrates is relatively more specific. It is widely accepted that the liver is a major site of glucuronidation. However, it is now clear that extrahepatic tissues are also involved in the conjugation of compounds to which these tissues are exposed [1,6,7]. Glucuronidation has been demonstrated in the kidney, gut, lung, skin, brain, adipose, thymus, prostate and breast [1,6,22,23]; and high levels of 5α-reduced C19 steroid glucuronides were found in the human prostate [24], breast cyst fluid [25] and ovary follicular fluid [26] suggesting that glucuronidation of 5α-reduced C19 steroids occurs in these tissues. Based on the expression of UGT2B transcripts in most if not all steroid target tissues [27–31], and the significant concentration of glucuronidated steroids found in these tissues and in the circulation of humans, it has been proposed that UGT enzymes can glucuronidate steroids and contribute to modulate steroid levels in extrahepatic steroid target tissues (Fig. 1) [29,32–34].

Earlier studies on steroid metabolism demonstrated that the major androgen metabolites found in the circulation of humans are glucuronidated androsterone (ADT) and 5α-androstane-3α, 17β-diol (3α-diol). It has been suggested that the plasma levels of 5α-reduced C19 steroid glucuronides, namely ADT-glucuronide (ADT-G) and 3α-diol-G, reflect the peripheral tissue conversion of adrenal and gonadal precursor C19 steroids to active androgens [35]. In addition, a recent study suggests that the serum levels of unconjugated androgens and estrogens are a poor indicator of total androgenic and estrogenic activities in men and women, whereas, the level of circulating glucuronidated androgen metabolites was shown to be correlated with the total androgen pool in men [36].

To further understand the role of steroid glucuronidation, novel cDNA clones encoding human and monkey UGT enzymes have been isolated and characterized. The recent results which will be discussed here, demonstrate the specificity of these novel enzymes for steroid substrates, and the expression of these UGT2B transcripts in specific extrahepatic steroid target tissues, which are consistent with a potential role of these proteins in steroid metabolism.

2. Results and discussion

2.1. Glucuronidation of steroids by UGT1 enzymes

Several UGT1 enzymes have recently been reported to glucuronidate steroids [16–20]. To date, the human UGT1 proteins which have been cloned and studied are UGT1A1 [37], UGT1A3 [19], UGT1A4 [37], UGT1A6 [38], UGT1A7 [39], UGT1A8 [20], UGT1A9 [40] and UGT1A10 [39,41]. In addition, the first exons in UGT1A2 (Genbank M84126), UGT1A11 (GenBank U39951) and UGT1A12 (GenBank U35992) have been proposed to yield pseudogenes, and do not encode functional proteins [8]. All of the UGT1 enzymes tested thus far, with the exception of UGT1A6, glucuronidate C18 steroids. The UGT1A3 [19], UGT1A4 [18] and UGT1A10 [20] enzymes can conjugate some C19 steroids, but their activities are apparently higher for estrogens and catechol estrogens. The cynomolgus monkey, which has been used frequently as an animal model for drug metabolism and steroidogenesis, also express UGT1A enzymes which have been shown to conjugate steroids (Vallée et al., unpublished results). The expression of UGT1 transcripts has been demonstrated in several different extrahepatic steroid target tissues including the skin, testis, ovary, breast and prostate (Albert et al., unpublished results). Although some of the UGT1 enzymes have overlapping specificity for steroid substrates, their expression is not ubiquitous nor concurrent in all the tissues tested. These data are consistent with UGT enzymes being involved in the elimination of endogenous and exogenous compounds to which human tissues are exposed.

2.2. Characterization of a human UGT2B androgen glucuronidation enzyme

Considering the significant concentration of glucuronidated ADT and 3α-diol found in the circulation of humans and in tissues such as the prostate, it was apparent that novel steroid-specific glucuronidation enzymes remained to be characterized. Chen et al. [42] demonstrated the activity of UGT2B15 on dihydrotestosterone (DHT) and 3α-diol, but this enzyme does not conjugate ADT. To isolate cDNA clones which encode steroid-specific glucuronidation enzymes (in particular ADT), libraries were constructed with mRNA from the liver, prostate and LNCaP cells. The LNCaP cell line is derived from a lymph node carcinoma of a prostate cancer patient, and as found with certain prostate cancers, the proliferation of these cells is androgen-dependent [33,43]. In addition, the LNCaP

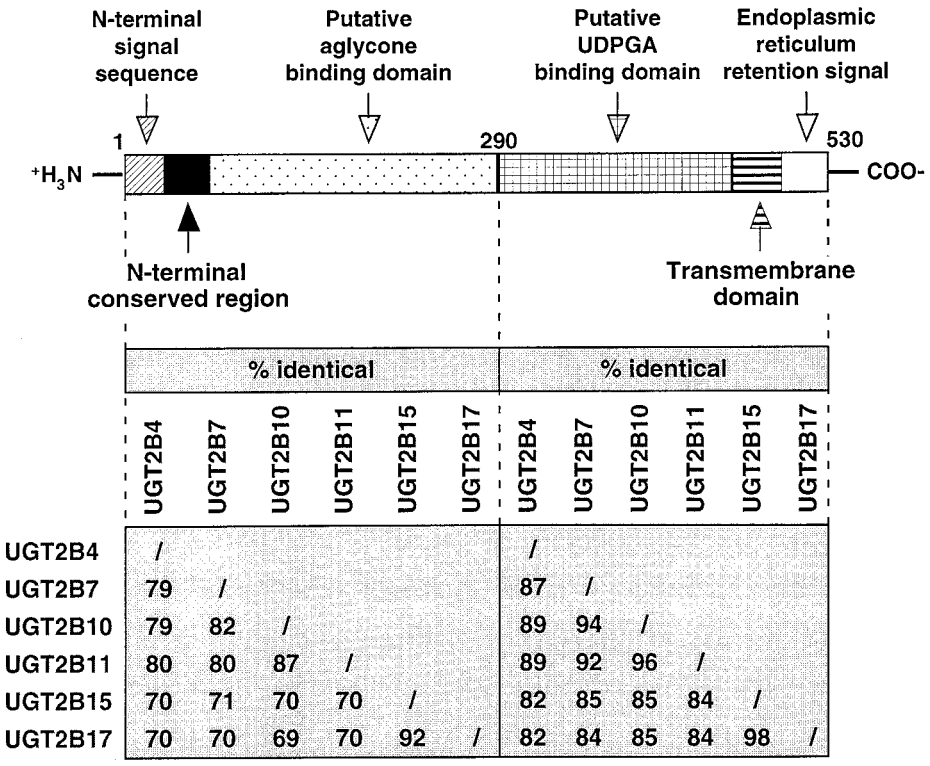

Fig. 2. Schematic representation of the structural and functional domains present in UGT enzymes. The amino acid sequence identity between the human UGT isoenzymes are as indicated, for the amino- (1–290) and the carboxyl-terminal (291–530) halves of the proteins.

cells were shown to possess enzyme activities capable of glucuronidating C19 steroids [28,29,32,33,43–46].

The UGT2B17 cDNA was isolated from both the LNCaP cell and human prostate libraries, which were screened with a combination of human probes synthesized from the full length UGT2B7, UGT2B10 and UGT2B15 cDNAs [29]. The UGT2B17 cDNA encodes a 530 amino acid polypeptide which contains the conserved putative signal sequence for the endoplasmic reticulum, and transmembrane domain characteristic of UGT2B proteins. The primary structure of UGT2B17 is highly homologous with other UGT2B enzymes and is 95% identical with UGT2B15 [29]. As seen with other UGT2B proteins, the amino-terminal half of UGT2B17, which has been proposed to contain the aglycone binding domain, is less homologous with

Table 1
Tissue distribution of human and monkey UGT2B transcripts[a]

Enzymes		Tissue						References
		Liver	Prostate	Testis	Mammary gland	Lung	Kidney	
Human	UGT2B4	+	+	+	+	+	+	[72]
	UGT2B7	+	–	–	+	+	+	[b]
	UGT2B10	+	+	+	+	+	+	[b]
	UGT2B11	+	+	–	+	+	+	[73]
	UGT2B15	+	+	+	+	+	+	[31]
	UGT2B17	+	+	+	+	+	+	[29]
Monkey	UGT2B9	+	+	+	–	–	–	[27]
	UGT2B18	+	+	–	–	–	+	[30]
	UGT2B19	+	+	–	+	–	–	[69]
	UGT2B20	+	+	+	+	–	+	[70]

[a] Total RNA isolated from the tissues as indicated, were analysed by RT–PCR using specific oligonucleotides for each UGT2B cDNA. The specificity of each RT–PCR product was confirmed by direct sequencing. + or – indicate the presence or absence of transcript, respectively.
[b] Turgeon et al., unpublished data.

the other UGT2B proteins than the carboxyl-terminal half of the protein, which has been suggested to contain the UDP-glucuronic acid binding domain (Fig. 2).

Stable expression of UGT2B17 in HK293 cells was used to demonstrate the activity on several C19 steroids which include testosterone (testo), DHT, 3α-diol and ADT. Kinetic studies show that UGT2B17 glucuronidates testo, DHT, 3α-diol and ADT with apparent K_m values of 3.4, 0.7, 1.0 and 0.4 μM, respectively. These results demonstrate the ability of UGT2B17 to conjugate androgens at the 17β-OH position (testo and DHT), as well as at the 3α-OH position (ADT). Similarly to other UGT2B enzymes isolated to date, UGT2B17 is highly reactive towards exogenous substrates, especially eugenol. It is clear that members of the UGT2B subfamily express overlapping substrate specificities; however, UGT2B7 [47,48] and UGT2B17 are presently the only human enzymes capable of glucuronidating ADT.

2.3. Tissue specific expression of UGT2B transcripts

To determine the tissue distribution of UGT transcripts, RT–PCR analyses demonstrated the expression of UGT2B15 and UGT2B17 in several extrahepatic steroid target tissues including the testis, breast and prostate (Table 1). Despite the wide distribution of these two transcripts, their expression is not concurrent in certain tissues such as in adipose, where UGT2B15 is expressed in the absence of UGT2B17 [49]. However, both proteins are expressed in the human prostate where androgens are physiologic regulators of cell differentiation and proliferation [50], and have profound effects on hormone dependent prostate cancer [51]. It is clear that UGT2B15 and UGT2B17 have overlapping substrate specificity and can both conjugate testo, DHT and 3α-diol however, it was uncertain if the role of the enzymes in the prostate is redundant. To address this question, an immunocytochemistry approach demonstrated cell type specific expression of different UGT2B proteins, and suggest that glucuronidation in specific cells of the prostate is catalyzed by specific enzymes, and not a pool of UGT proteins with overlapping activities. Recently, El-Alfy et al. [52] demonstrated the expression of 17β-HSD type 5 and 3β-HSD predominantly in the basal cells of the human prostate epithelium, whereas the androgen receptor was localized in luminal cell nuclei. A two-cell mechanism of androgen synthesis in the human prostate was suggested; testosterone is synthesized in the basal cells before diffusing into the luminal cells where transformation into DHT and binding to the androgen receptor occurs. The co-expression of UGT2B17 with the steroidogenic enzymes 17β-HSD and 3β-HSD in basal cells is consistent with this protein's involvement in steroid metabolism. As well, the co-expression of UGT2B15 with 5α-reductase and the androgen receptor suggests a role of this enzyme in regulating the elimination of DHT and its metabolites from the luminal cells. It may also be possible that UGT2B15 is involved in the androgen response by glucuronidating DHT, and abolishing its interaction with the androgen receptor.

2.4. Differential regulation of UGT2B expression

The expression of different UGT enzymes in a cell type specific manner, and the co-localization with different steroidogenic enzymes indicate that UGT enzymes play distinct physiological roles despite having similar substrate specificities. To study the regulation of UGT expression in response to physiological effectors, and to further examine the relationship between UGT2B15 and UGT2B17, their regulation of expression was examined in LNCaP cells. Treating the cells with the androgen DHT or epidermal growth factor (EGF) decreased the steady-state levels of UGT2B transcripts as seen by Northern blots, and was accompanied by a decrease of DHT and ADT glucuronidation [32,33,43]. As well, treatment with both DHT (0.5 nM) and EGF (10 ng/ml) caused a greater decrease of DHT glucuronidation and UGT2B mRNA levels than when the cells were treated with either compound alone. To more specifically determine which UGT2B transcripts were regulated, RNase protection analyses demonstrated a decrease in the level of UGT2B17 transcript in the presence of DHT and EGF; however, these effectors did not change the level of UGT2B15 mRNA. Western blot analysis, using the antisera (EL95) specific for UGT2B17, also demonstrated the effect of DHT and EGF at the protein level, where the decrease of UGT2B17 protein by 75% correlated with the decreased production of DHT-G by 73% [32]. To determine if the inhibition of UGT2B17 expression and DHT-G formation by EGF is mediated by a known physiological signal transduction pathway and not due to a non-specific effect, experiments were performed to specifically block the action of EGF. Treating the cells with tyrphostin A46, which is a specific inhibitor of the EGF-receptor tyrosine kinase [53], partially reversed the inhibitory effect of EGF on UGT2B17 expression and DHT glucuronidation. As well, incubating the cells with a specific antibody against the EGF receptor [54], which was previously shown to block EGF binding, also reduced the inhibitory effect of EGF on DHT glucuronidation. Although the inhibitory effect of androgens and EGF on UGT enzymes may be mediated through different signal transduction pathways, there is evidence of cross-talk between these two pathways [55]. As an example, the recent observation that an androgen response can be activated by EGF in an androgen-

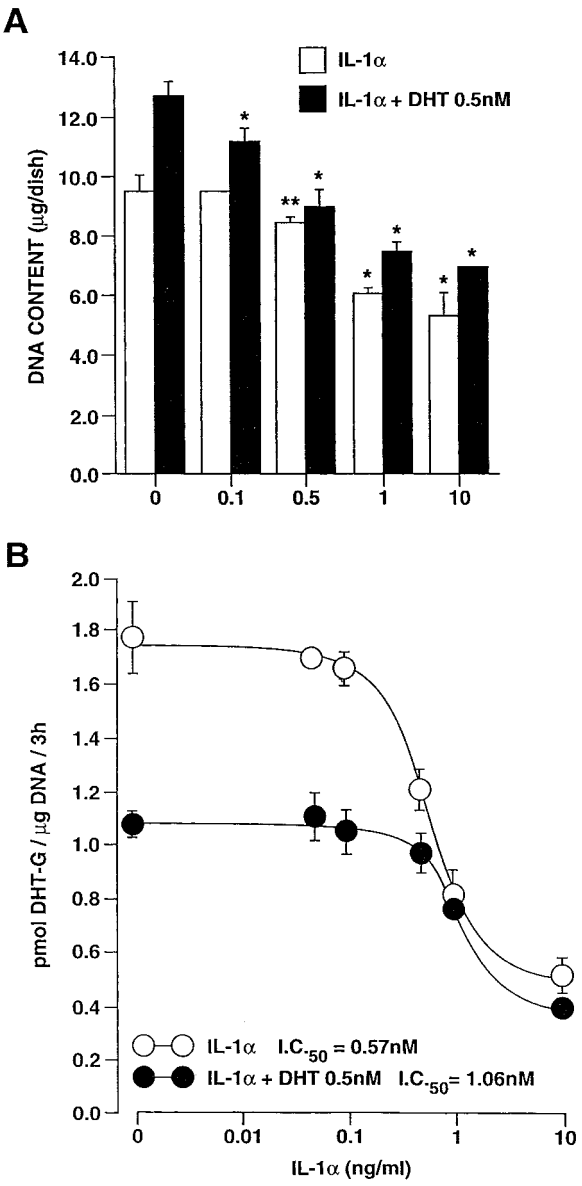

Fig. 3. Effects of IL-1α on LNCaP cell proliferation and DHT glucuronidation. The proliferation (A) and UGT activity (B) of human prostatic cancer LNCaP cells were determined after continuous exposure (6 days) to increasing concentrations of IL-1α (1–10 ng/ml) in the presence and the absence of DHT (0.5 nM). Cells were initially plated at a density of 45000 cells/well. DNA content was measured after determination of DHT-G formation. The data are the mean ±SEM from 3 separate experiments each consisting of triplicate determinations. $^{**}=p=0.01$ and $^{*}=p=0.001$; treatments versus control without IL-1α.

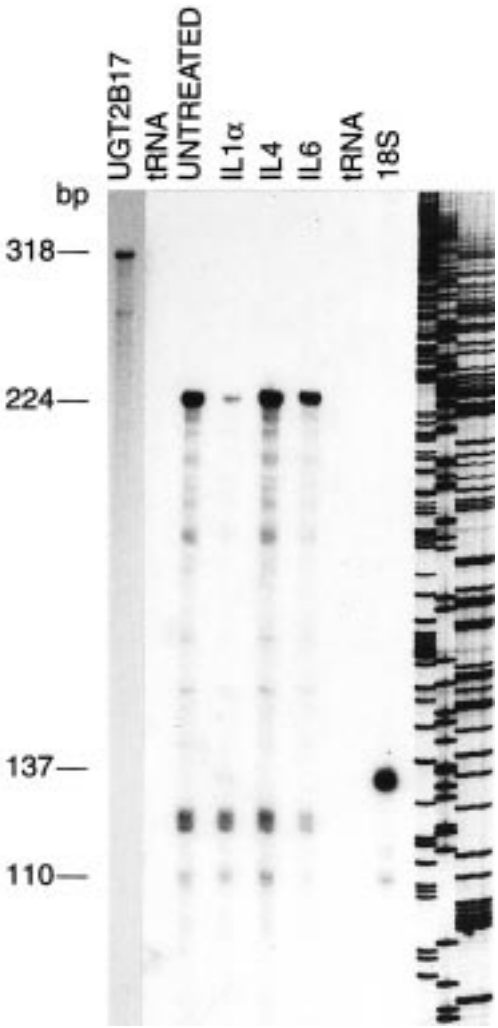

Fig. 4. RNase protection analyses of UGT2B17 transcript in LNCaP cells treated with IL-1α, IL-4 and IL-6. 25 μg of total RNA isolated from untreated and treated LNCaP cells were hybridized to a UGT2B17 cRNA probe. The UGT2B17 probe of 318 bp protected a fragment of appropriate length (224 bp). The integrity of the RNA samples was assessed using a 137 bp 18 S cRNA probe and protected a fragment of 110 bp in each RNA preparation. The quantity of 18 S RNA was used to normalize the amount of RNA in the samples. The sizes of the probe and protected fragments are indicated on the left. The sequence shown on the right was used to confirm the sizes of the cRNA probes and protected fragments. All samples were separated on a denaturing 6% polyacrylamide gel.

depleted environment strongly suggests the interaction of growth factors with the androgen-signal transduction cascade in prostatic tumor cells [56]. Thus far, it is apparent that both DHT and EGF are capable of inhibiting androgen glucuronidation via different mechanisms, and appear to be synergistic. The expression of EGF and the EGF-receptor has been demonstrated in the human prostate and in LNCaP cells [57–60], and cell proliferation was stimulated both in vivo and in vitro by several growth factors, including EGF [58,60–64]. These factors involved in the regulation of tumor proliferation and progression, can regulate UGT2B17 expression. The inhibitory effect of androgens and growth factors on androgen glucuronidation may enhance the proliferation of androgen-dependant tumors since a decrease in the glucuronidation of DHT or its 5α-reduced metabolites can favor an accumulation of DHT.

As was found with EGF, the treatment of LNCaP cells with interleukin-1α (IL-1α) also decreased the glu-

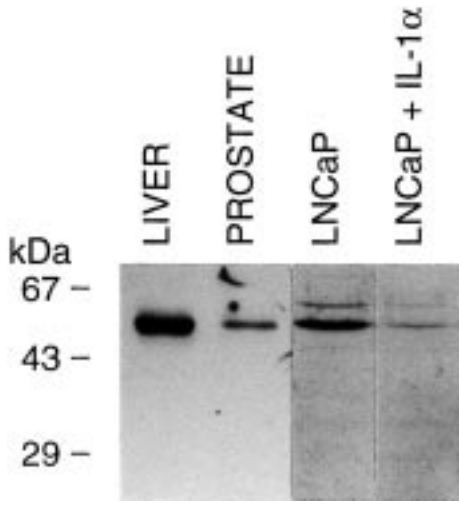

Fig. 5. Immunoblot analysis of UGT2B17 protein following treatment of LNCaP cells with IL-1α. Microsomal proteins from human liver (2 μg), prostate (10 μg), untreated LNCaP cells and IL-1α treated LNCaP cells (10 μg) were separated by 12% SDS–PAGE; and transferred onto nitrocellulose membrane for analysis with the anti-UGT2B17 (EL95) antibody.

curonidation of androgens (Fig. 3), and specifically inhibited the levels of UGT2B17 transcript (Fig. 4) and protein (Fig. 5) with no effect on the levels of UGT2B15. As well, treatment with IL-4 or IL-6 had no effect, thus indicating the specificity of IL-1α on the expression of UGT2B17 [65]. The differential response of UGT2B15 and UGT2B17 to physiological effectors is consistent with the enzymes playing different roles. The correlation between inhibition of glucuronidation with the reduction of UGT2B17 expression indicates the potential role of this enzyme in steroid metabolism in LNCaP cells and the human prostate.

Another difference observed between UGT2B15 and UGT2B17 is their relative stabilities. Using HK293 cells which stably express exogenous UGT2B15 or UGT2B17, the half-life of UGT2B17 activity was estimated to be approximately 3 h, whereas the activity of UGT2B15 was unchanged after 24 h [32]. The more rapid turnover of UGT2B17 indicates that the observed regulation by DHT and EGF to decrease the level of UGT2B17 mRNA in prostate cells would lead to a rapid reduction in the level of UGT2B17 protein, and potentially alter the response of androgen in a tissue such as the prostate [32].

To determine if the inhibitory effects of androgens, EGF and IL-1α on glucuronidation are due to decreased transcription, the *UGT2B17* gene was isolated. This gene is comprised of 6 exons spanning approximately 30 kb, and was mapped to human chromosome 4q13 [66]. The 5′-flanking region of *UGT2B17* was demonstrated to confer promoter activity when transiently transfected into LNCaP cells. Treatment of the cells with R1881, EGF or IL-1α led to a 50% inhibition of reporter gene expression, thus demonstrating their effect at the level of transcription (Fig. 6). The 5′-flanking region of *UGT2B17* contains concensus binding sites for several transcription factors including AP-1, C-EBP, NF-κB, Pbx-1 and Oct-1; however, promoter deletion analysis suggests that inhibition of *UGT2B17* in response to the effectors involves AP-1 [65,66].

2.5. The cynomolgus monkey as an in vivo model to study steroid glucuronidation

To obtain an in vivo system in which to study the

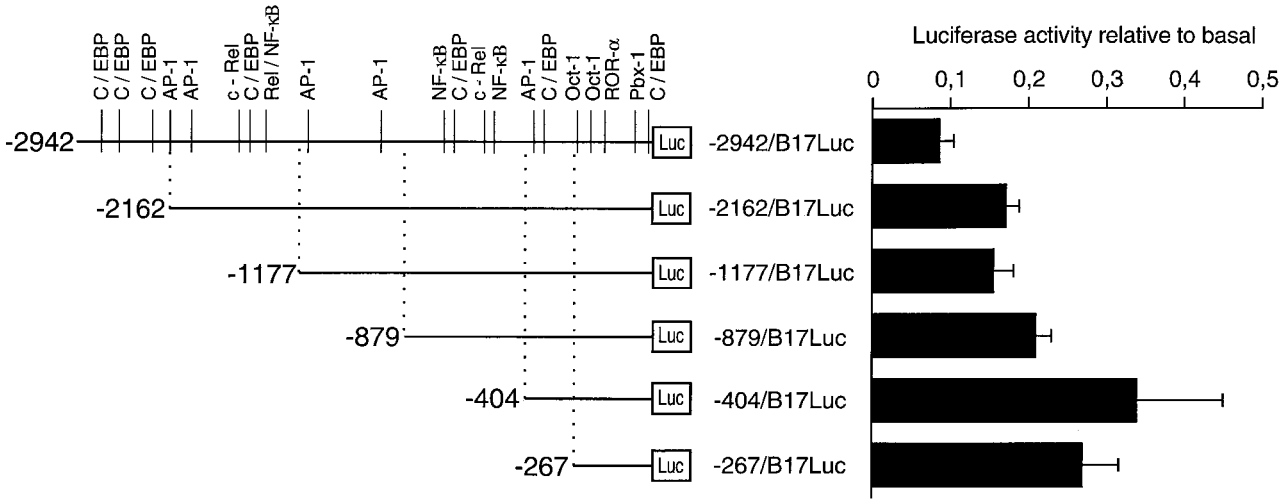

Fig. 6. Effect of IL-1α on UGT2B17 gene promoter activity. Equimolar amounts of each reporter construct was transiently transfected into LNCaP cells in the presence or absence of IL-1α (10 ng/ml). Following 48 h of incubation, the cells were harvested and luciferase activity determined. Each value represents the mean ±SEM of three independent experiments each performed in duplicate. All the experiments were normalized with cotransfection of the CMV-βgal vector.

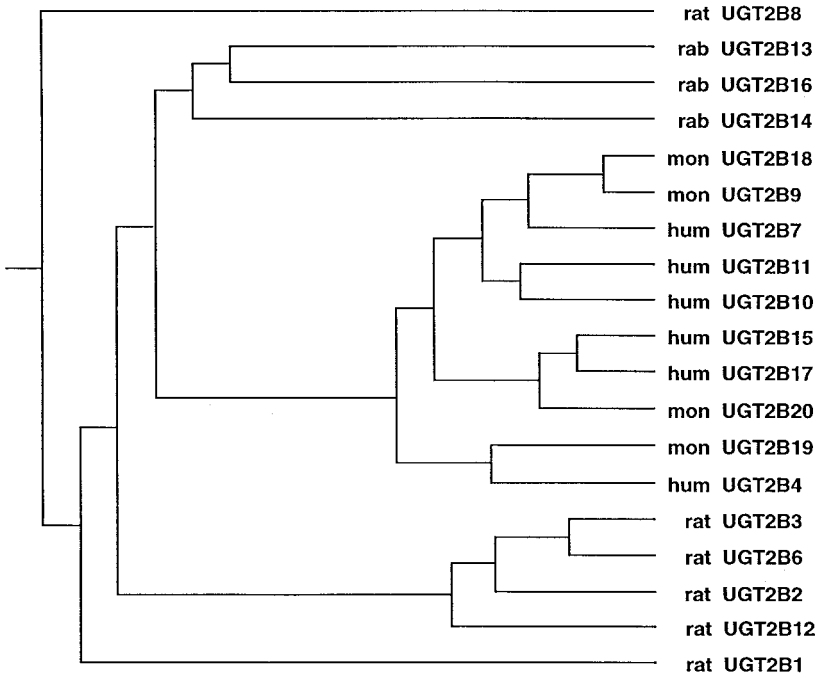

Fig. 7. Dendogram of homology between the UGT2B enzymes from rat, rabbit (rab), human (hum) and monkey (mon). The amino acid sequences were obtained from the GenBank data base.

role of steroid glucuronidation, it was previously ascertained that simians may be the most appropriate animal model [67]. Unlike some common laboratory mammals such as the rat and mouse, which have different patterns of steroidogenesis than in humans, simians express the same enzymes involved in steroid synthesis, and have similar patterns of tissue specific steroidogenesis. In addition, the monkey and human both secrete DHEA from their adrenals as the major precursor steroid for androgens and estrogens [68]; and a comparison of the circulating levels of 5α-reduced C19 steroid glucuronides among mammalian species, showed that human and monkey are unique in having high levels of circulating ADT-G and 3α-Diol-G [30,67]. The homology between the human and monkey UGT2B proteins was initially demonstrated by Northern blot analysis with human cDNA probes, which hybridized to simian transcripts of the appropriate size [27]. As well, polyclonal antibodies raised against human UGT2B proteins also recognize appropriate size proteins in monkey tissue extracts [27]. As found in humans, extrahepatic steroid target tissues, which include the prostate, testis, skin and breast, of the monkey also express steroid conjugating UGT transcripts (Table 1). All these data indicate that the monkey is an appropriate animal model for studying the role of steroid glucuronidation in extrahepatic tissues [67].

To isolate simian UGT2B cDNA clones for further analyses, liver and prostate cDNA libraries were made with mRNA isolated from the cynomolgus monkey [27]; and were screened with a combination of human probes synthesized from the full length cDNAs of UGT2B7, UGT2B10 and UGT2B15. Twenty positive cDNAs were isolated, and nucleotide sequence analysis revealed five novel clones including UGT2B9 [27], UGT2B18 [30], UGT2B19 [69] and UGT2B20 [70]. The primary structures of the simian UGT2B proteins are highly homologous to the human enzymes and share 62–92% identity (Fig. 7). As expected, the amino terminal halves of the protein are less homologous than the carboxyl terminal halves. The monkey enzymes can conjugate C19 steroids as seen with the human proteins; however, the overall substrate specificity of a given simian enzyme is slightly different than the human proteins which make it difficult to classify the enzymes as true orthologues. UGT2B9, UGT2B19 and UGT2B20 are active on C18 and C19 steroids, while UGT2B18 glucuronidates only C19 steroids containing an hydroxyl group at position 3α of the molecule. The ability of UGT2B18 to conjugate ADT and not testosterone or DHT may help explain the relatively high level of ADT-G found in the circulation in comparison to testosterone-G and DHT-G. It is interesting that the rat UGT2B2 enzyme, which has the same specificity for ADT as UGT2B18, has been characterized, whereas a human enzyme with the same characteristics has not been isolated. Based on the similar patterns of steroid metabolism between the primates, and the significant levels of glucuronidated

ADT and 3α-diol found exclusively in primates, it would be reasonable to speculate that a human orthologue of UGT2B18 remains to be isolated.

Based on the high sequence homology (92% identical primary structure) and similar substrate specificity, UGT2B20 [70] is an apparent simian orthologue of the human UGT2B15 [31,42,71] enzyme. Kinetic analyses of the human and monkey UGT2B enzymes demonstrate conjugation of steroids with K_m values in the low μM range which is consistent with these proteins having a physiological role in steroid glucuronidation. The UGT2B20 transcript was found in several extrahepatic steroid target tissues, such as the epididymis, adrenals, prostate, breast and kidney. However, expression was not detected in the heart, spleen, bladder, thyroid, colon, pancreas, gall bladder and stomach [70]. Expression of UGT2B transcripts in peripheral steroid target tissues indicates that these tissues are involved not only in the production of steroid hormones [68], but are also capable of conjugating steroid metabolites, thus suggesting the potential role of UGT enzymes in steroid catabolism in a tissue.

From the results obtained thus far, it is clear that extrahepatic steroid target tissues express steroid conjugating UGT1A and UGT2B enzymes. In a tissue such as the human prostate, several different UGT2B proteins are expressed including UGT2B15 and UGT2B17. Despite the expression of several UGT2B enzymes with overlapping steroid specificities, the proteins are expressed specifically in different cell types in the prostate, thus suggesting that the different enzymes have separate physiological roles. The observation that UGT2B15 and UGT2B17 respond differentially to physiologic effectors, and have very different protein stabilities, further suggest their different roles in response to the physiological requirements of a given cell or tissue. It is most probable that additional steroid glucuronidation enzymes remain to be characterized, which would help to better understand the role of steroid glucuronidation. As well, the characterization of these enzymes in the monkey will provide a model in which to study in vivo and further understand the physiological role and relevance of the individual UGT proteins.

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