Isolation and Characterization of a Novel cDNA Encoding a Human UDP-Glucuronosyltransferase Active on C19 Steroids*

(Received for publication, March 11, 1996, and in revised form, May 13, 1996)

Martin Beaulieu‡, Éric Lévesque‡‡, Dean W. Hum, and Alain Bélanger¶
From the Medical Research Council Group in Molecular Endocrinology, CHUL Research Center, Laval University, Québec G1V 4G2, Canada

To isolate cDNA clones encoding novel UGT2B enzymes, human prostate and LNCaP cell cDNA libraries were screened using a pool of steroid-specific UGT2B cDNA probes. In approximately 10⁶ recombinants, we isolated 3 cDNA clones of 2.1 kilobases that encode a novel UGT2B enzyme. UGT2B17 is 95% identical with UGT2B15 and 91% identical with UGT2B8. Primary structure analysis of UGT2B17 based on the nucleotide sequence revealed a putative amino-terminal membrane insertion signal peptide, a carboxyl-terminal membrane-spanning region, and three potential asparagine-linked glycosylation sites. UGT2B17 cloned in the pBK-CMV expression vector was transfected into HK293 cells to obtain a stable clonal cell line expressing a high level of the active 53-kDa UGT2B17 enzyme. Of the over 60 endogenous and exogenous substances tested, 25 compounds revealed reactivity. The major substrates are eugenol > 4-methylumbelliferone > dihydrotestosterone > androstan-3α,17β-diol (3α-diol) > testosterone > androstenedione (ADT). The apparent Km values obtained with tritiated steroids in intact cells were 0.4 μM for ADT, 0.7 μM for dihydrotestosterone, 1.0 μM for 3α-diol, and 3.4 μM for testosterone. Southern blot analysis of reverse transcription-polymerase chain reaction products revealed expression of UGT2B17 mRNA in various tissues including the liver, kidney, testis, uterus, placenta, mammary gland, adrenal gland, skin, and prostate. UGT2B17 is the first human uridine diphosphoglucuronosyltransferase enzyme expressed in extrahepatic tissues to have a specificity for ADT as well as testosterone, dihydrotestosterone, and 3α-diol.

Uridine diphosphoglucuronosyltransferases (UGTs; EC 2.4.1.17) represent a family of enzymes that catalyze the transfer of glucuronic acid from uridine diphosphoglucuronic acid to a wide variety of lipid-soluble drugs, environmental chemicals, and endogenous substances such as bilirubin, steroid hormones, and thyroxine (1–6). Although the liver and kidney are known to be major sites of glucuronidation that eliminate glucuronic acid derivatives from the body, accumulating evidence also indicates the presence of UGT activities, particularly for steroid hormones, in several tissues such as the prostate (7–9), testis (9), skin (10), breast (11), brain (12), and ovary (13). In addition, steroid UGT activities were also observed in breast and prostate tumor cell lines such as MCF-7, ZR-75–1, and LNCaP (7, 8, 14–16). The presence of steroid UGTs in several extrahepatic tissues suggests that these steroid-conjugating enzymes may be involved in facilitating excretion of steroids into the circulation.

The concept of regulating tissue substrate concentration by UGT enzymes was previously suggested by Lazard et al. (17) who reported that low molecular mass volatile molecules can be glucuronidated by specific UGTs present in the olfactory epithelium. Although it was first observed that excess odorant is converted by cytochrome P450s to polar compounds and excreted, these authors demonstrated that glucuronidation of hydroxylated intermediates also plays a major role in eliminating these products from the olfactory epithelium, thus indicating that UGTs provide an effective mechanism of removing excess odorant leading to signal termination (18). The action of steroids depends upon several factors including the steroid receptor machinery, the concentration of steroids, and finally the metabolism of steroids in the target tissue. The presence of an irreversible enzymatic step such as glucuronidation in the pathway of steroid metabolism may contribute to the regulation of tissue steroid concentrations.

Dihydrotestosterone (DHT), the major androgen in the human prostate, plays an important role as modulator of several parameters of this tissue including its growth and secretion of a variety of proteins (19–21). Several DHT metabolizing enzymes such as 3β-hydroxyysteroid dehydrogenase (3β-HSD), 3α-hydroxysteroid dehydrogenase (3α-HSD) and 17β-hydroxysteroid dehydrogenase (17β-HSD) are present in the prostate and induce the formation of several 5α-reduced C19 steroid metabolites such as androsterone (ADT) and androstane-3α,17β-diol (3α-diol) (22–24). Although the metabolism of DHT into other 5α-reduced C19 steroids markedly reduces the androgenic activity due to their low affinity for the androgen receptor, conversion of DHT by 17β-HSD, 3β-HSD, and 3α-HSD does not irreversibly metabolize DHT. Data obtained by our group suggest that glucuronidation is involved in deactivation of androgens in the human prostate (7, 15). In agreement with the high prostate concentrations of 3α-diol glucuronide (3α-diol-G), the presence of UGT2B15, a UGT enzyme that specifically glucuronidates DHT and 3α-diol at position 17β-hydroxy, was recently confirmed in this tissue (8, 9).

Interestingly, it has been postulated that the circulating levels of 5α-reduced C19 steroid glucuronides, such as 3α-diol-G and ADT-G, may reflect the metabolism of androgens in a large

---

*This work was supported by the Medical Research Council (MRC) of Canada, the Fonds de la recherche en Santé du Québec, and En- dorcherche. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1744 solely to indicate this fact.

‡ These authors contributed equally to this work.

§ Holder of a scholarship from the MRC of Canada.

¶ To whom correspondence should be addressed: Molecular Endocrinology Laboratory, CHUL Research Center, 2705 Laurier Blvd., Que- bec, G1V 4G2, Canada. Tel.: 418-654-2296; Fax: 418-654-2761; E-mail: Alain.Belanger@crchul.ulaval.ca.

1 The abbreviations used are: UGT, uridine diphosphoglucuronosyltransferase; DHT, dihydrotestosterone; HSD, hydroxysteroid dehydro- genase; ADT, androstenedione; 3α-diol, androstane-3α,17β-diol; UDPGA, UDP-glucuronic acid; HPLC, high pressure liquid chromatography; PCR, polymerase chain reaction; RT-PCR, reverse transcription-polymerase chain reaction; bp, base pair(s).
number of androgen target tissues (7, 25, 26). In both men and women, the levels of ADT-G in the circulation exceed by approximately 5-fold the levels of 3α-diol-G. Since UGT2B15 cannot glucuronidate 3α-hydroxy steroids such as ADT, it must be assumed that a second uncharacterized UGT2B enzyme is present. Using LNCaP cells, an epithelial cell line originating from a lymph node carcinoma of the prostate (27), we recently demonstrated that both ADT and 3α-diol can be rapidly converted to glucuronidate derivatives, and competition studies of UGT activity using several steroids suggest the presence of at least two different UGTs (8, 15, 16).

Based on evolutionary divergence of the isolated cDNA clones, Burchell et al. (28) suggested a nomenclature to classify the UGT superfamily into two families, UGT1 and UGT2. The members of the UGT1 family are derived by alternative splicing of a single gene, and they are generally known to glucuronidate planar and bulky phenol substrates and bilirubin, whereas some members of the human UGT1 family can conjugate estrogens. Enzymes of the UGT2 family are divided in two subfamilies: UGT2A enzymes, which are encoded by genes expressed in the olfactory epithelium, and UGT2B enzymes that catalyze the glucuronidation of bile acids, C19 steroids, C18 steroids, fatty acids, carboxylic acids, phenols, and carcinogens such as benzoquinone and 2-acylaminofluorene (28). Comparison of the cDNA sequences of UGT2 enzymes suggests that UGT2 enzymes are encoded by independent genes. However, the chromosomal localization of human UGT2B4, UGT2B9, and UGT2B15 shows that these genes are clustered within a 195-kilobase region of chromosome 4q13 (29). As in the UGT1 family, there is an overlap between the substrate specificity of UGT2B enzymes (30). In human liver, seven cDNAs from the UGT2B subfamily have been isolated. Among these, UGT2B4, UGT2B7, UGT2B8, and UGT2B11 are specific for estriol and/or 3,4-catecholestrogens (31–35), and UGT2B15 glucuronidates DHT and 3α-diol (9, 36).

In the present study we have isolated and characterized a novel human cDNA clone isolated from LNCaP cell and human prostate libraries. The cDNA encodes a UGT enzyme (UGT2B17) capable of conjugating C19 steroids at the 3α-hydroxyl and 17β-hydroxy groups. This enzyme is responsible for the conversion of ADT into ADT-G, which is the predominant 5α-dihydro derivative, and the extent of glucuronidation was assessed by PhosphorImager analysis. Enzyme assays were performed using [14C]UDP-glucuronic acid (UDPGA) and 3H-testosterone (90 Ci/mmol), [3H]Androsterone (59 Ci/mmol), [9,11-3H]Androstane-3β,17β-diol (56 Ci mmol), and [14C]UDP-glucuronic acid (285 mCi/mmol) were obtained from Dupont NEN. [1,2,6,7-3H]Dihydrotosterone (47 Ci/mmol), [1,2,6,7-3H]testosterone (90 Ci/mmol), α-[32P]dCTP (3000 Ci/mmol), and [α-32P]UTP (3000 Ci/mmol) were from Amerham Corp. Geneticin (G418) and Lipofectin were obtained from Life Technologies, Inc. Protein samples were obtained from Prostate, bladder, mammary gland, and prostate, thus clearly establishing its expression in extrahepatic steroid target tissues.

**Experimental Procedures**

**Materials**—UDP-glucuronic acid and all aglycons were obtained from Sigma and ICN Pharmaceutical Inc. (Montreal, Canada). Radioinert steroids were purchased from Steraloids Inc. (Wilton, NH). [9,11-3H]Androsterone (59 Ci/mmol), and [14C]UDP-glucuronic acid (285 mCi/mmol) were obtained from Dupont NEN. [1,2,6,7-3H]Dihydrotosterone (47 Ci/mmol), [1,2,6,7-3H]testosterone (90 Ci/mmol), α-[32P]dCTP (3000 Ci/mmol), and [α-32P]UTP (3000 Ci/mmol) were from Amerham Corp. Geneticin (G418) and Lipofectin were obtained from Life Technologies, Inc. Protein samples were obtained from Prostate, bladder, mammary gland, and prostate, thus clearly establishing its expression in extrahepatic steroid target tissues.

**Human RNA Isolation**—Total RNA was isolated from human liver, adipose tissue, skin, placenta, benign prostate hyperplasia tissue, and LNCaP cells according to the Tri reagent acid phenol protocol as specified by the supplier (Molecular Research Center Inc., Cincinnati, OH). The mRNA obtained from benign prostate hyperplasia tissue and LNCaP cells were affinity-purified by chromatography through oligo(dT)-cellulose (Pharmacia).

**cDNA Isolation**—Affinity-purified benign prostate hyperplasia tissue and LNCaP cell mRNAs were used to construct cDNA libraries in the ZAP Express vector as specified by the supplier (Stratagene, La Jolla, CA). Both libraries were not amplified for screening where the filters were prehybridized in 40% formamide, 5× Denhardt’s solution, 5× SSPE, 0.1% SDS, and 100 mg/ml salmon sperm DNA for 4 h at 42 °C. The hybridization was performed in the same solution for 16 h at 42 °C with 2.0×10⁶ cpm/ml of a pool of probes derived from the UGT2B7, UGT2B10, and UGT2B15 cDNAs (8). cDNA probes were radiolabeled by the random primer technique in the presence of [α-32P]dCTP (39). The filters were washed twice in 2× SSC, 0.1% SDS at 42 °C for 15 min and exposed for 2 days at ~80 °C on XAR5 film with an intensifying screen (Eastman Kodak Co.).

After screening approximately 1×10⁶ recombinants, 30 positive clones were isolated from the LNCaP cell library and 5 from the benign prostate hyperplasia tissue cDNA library. UGT2B17 cDNA clones were isolated from both libraries and were sequenced in both directions using specific UGT oligonucleotide primers.

**In Vitro Transcription/Translation Assay**—The entire UGT2B17 cDNA in the pBK-CMV vector (Stratagene, La Jolla, CA) (pBK-CMV-UGT2B17) was transcribed using T3 RNA polymerase and translated using the TNT-coupled rabbit reticulocyte lysate system from Promega Corp. (Madison, WI). The protein product was separated on 10% SDSPAGE (Novex) and exposed on a phosphoimager film for 2 h.

**Stable Expression of UGT2B17**—HK293 cells were grown in Dulbecco’s modified Eagle’s medium containing 4.5 g/liter glucose, 10 mM HEPES, 110 µg/ml sodium pyruvate, 100 IU/ml penicillin, 100 µg/ml of streptomycin, and 10% fetal bovine serum in a humidified incubator, with an atmosphere of 5% CO2 at 37 °C. 5 µg of pBK-CMV-UGT2B17 were used to transfect HK293 cells using Lipofectin according to the manufacturer’s instructions (Life Technologies, Inc.). 48 h posttransfection, stable transfectants were selected in media containing 800 µg/ml G418. After five rounds of selection, a clonal cell line stably expressing a high level of UGT2B17 was isolated.

**Glucuronidation Assay Using Cell Homogenates**—HK293 cells expressing UGT2B17 were suspended in Tris-buffered saline (42) containing 0.5 mM ethylenediamine and homogenized using a Brinkman polytron. Enzyme assays were performed using [14C]UDP-glucuronic acid (UDPGA), 500 µM of the various aglycons, and 150 µg of protein from cell homogenates in 50 mM Tris-HCl (pH 7.5), 10 mM MgCl2, 100 µg/ml phosphatidylcholine, and 8.5 mM saccharolactone in a final volume of 100 µl. The enzyme assays were terminated by adding 100 µl of methanol to the tubes and the tubes were centrifuged at 14,000 × g for 1 min to remove the precipitated proteins. 100 µl of the aqueous phase were applied onto TLC plates (0.25-mm-thick silica gel 60 F254 S) (EM Science, Gibbstown, NJ) and chromatographed in a solvent of toluene: methanol:acetic acid (7:3:1). The TLC plates were exposed for 4 days, and the extent of glucuronidation was assessed by PhosphorImager (Molecular Dynamics).

To screen for substrates that react with UGT2B17, the assays were conducted using 6 µM of [14C]UDPGA and 94 µM of unlabeled UDPGA for 15 min at 30 °C to determine enzyme activity. The enzyme reaction is linear for 30 min under these conditions where the Km of UDPGA is 200 µM. Glucuronidation activity was not detected in nontransfected HK293 cells.

**Km Determination in Intact HK293 Cells**—Km determinations were performed by incubating intact HK293 cells with steroid substrates. HK293 cells stably expressing UGT2B17 were plated at a density of 1×10⁶ cells/ml in 24-well plastic plates, and to remove steroids found in fetal bovine serum these experiments were performed in 2% (v/v) fetal bovine serum that had been treated twice with dextan-coated charcoal. Cells were incubated with 50 nm to 5.0 µM of radioinert and 10–50 nm of radiolabeled ADT, 3α-diol, DHT, or testosterone for 5 h at 37 °C. The medium was then removed and analyzed for glucuronide conjugates by organic extraction and total counting as described previously (16). The steroids and their conjugated metabolites were also verified by HPLC as described previously (15). The data obtained were normalized by RNA content quantitated by fluorometric assay with 3,5-diaminobenzoic acid (39).

**Ribonuclease Protection Assay**—To generate a probe specific for UGT2B17, the pBK-CMV-UGT2B17 construct was linearized by EcoRI digestion.
digested, and a radiolabeled cRNA probe of 318 bases from nucleotide 1394 to 1629 of the 3'-untranslated region, including 53 bases from the vector, was generated using T7 RNA polymerase and [γ-32P]UTP as described in the MAXIscript kit (Ambion, Austin, TX). For all the ribonuclease protection assays, 25 μg of total RNA was hybridized with 250,000 cpm of the appropriate cRNA probe for 16 h at 42 °C. cRNA-RNA hybrids were digested with 0.5 units of RNase A and 20.0 units of RNase T1 for 30 min at 37 °C, and the protected products were analyzed on a 7.5% urea, 6% polyacrylamide gel.

Reverse Transcriptase Polymerase Chain Reaction Analysis (RT-PCR)—The reverse transcriptase reaction was performed using 10 μg of total human RNA. The reaction was carried out using 200 units of Moloney murine leukemia virus reverse transcriptase in 125 μM of dNTPs, 10 mM Tris-HCl, pH 8.3, 50 mM KCl, 1.5 mM MgCl2, 32.4 units of RNase Guard and 100 pmol of the antisense primer in a total volume of 20 μl for 90 min at 37 °C. After inactivation of the reverse transcriptase for 5 min at 100 °C, one-fourth of the reverse transcriptase product was used as a template in a PCR containing 10 mM Tris-HCl, pH 8.3, 50 mM KCl, 1.5 mM MgCl2, 0.1 mM dNTP, and 1.25 units of AmpliTaq DNA polymerase in a total volume of 100 μl. The reaction was carried out using 100 pmol of the specific sense primer 5'-CTATGGTACTCTTGAGTCTCTATAGAT-3' and antisense primer 5'-ATAATTCTCATGATATCTCTTATAGAT-3'. The PCR was performed for 35 cycles (1 min at 94 °C, 1 min at 55 °C, 1 min at 72 °C), after which one-fifth of the PCR product was electrophoresed on a 1% agarose gel and transferred onto a nylon membrane for Southern blot analysis. Hybridization was carried out with the radiolabeled full-length UGT2B17 cDNA probe, and the blot was washed at high stringency conditions in 0.1 X SSC, 0.1% SDS at 60 °C for 30 min and exposed for 4 h on an XAR hyperfilm. All PCR reactions were controlled using specific oligonucleotides for glyceraldehyde-3-phosphate dehydrogenase. The identity of all PCR products was verified by direct sequencing.

Plasma Levels of ADT-G and 3α-Diol-G—Subjects were respondents to the Laval University Prostate Cancer Detection Program randomly selected from the electoral rolls of Quebec City and vicinity as described previously (24). The sample of 104 men from 40 to 45 years of age was randomly chosen, and none took medication known to affect the pituitary-adrenal or pituitary-testicular axis. Plasma ADT-G and 3α-diol-G were determined as described previously (24).

RESULTS

Isolation of a Novel UGT2B cDNA—Based on our previous observation that a significant amount of ADT-G is found in the human prostate (38), it was apparent that this tissue expresses a UGT capable of glucuronidating C19 steroids in the 3α-hydroxyl position. To isolate the cDNA encoding the C19 steroid UGT isoform we screened an LNCaP cell and a human prostate cDNA library. Due to the high homology between the UGT isoenzyme we screened an LNCaP cell and a human prostate cDNA library. Due to the high homology between the UGT2B cDNA clones isolated to date, which encode steroid-specific UGT enzymes, the libraries were screened using a pool of radiolabeled cDNA probes synthesized from the human UGT2B7, UGT2B10, and UGT2B15 cDNAs. After screening approximately 106 recombinants, 2 cDNA clones were isolated from the LNCaP cell library, and a single cDNA was isolated from the prostate library, which encoded the novel UGT2B17 enzyme. All three clones were sequenced in both directions and were found to be identical except for the length of their 5'-untranslated region and the number of adenine residues in the poly(A) tail. The longest UGT2B17 cDNA isolated was 2107 bases in length and contains an open reading frame of 1590 bases, 530 amino acids, 5α- and 5β-hydroxy androgens (Fig. 4). Of the 29 amino acids that are nonidentical between UGT2B15 and UGT2B17, 23 of the differences are found in the amino-terminal half of the protein. The rat 3α-hydroxysteroid UGT (UGT2B2) (46), which is principally active on ADT and etiocholanolone, shares only 65% identity with UGT2B17, whereas the human estrogen specific UGT isoforms UGT2B4, UGT2B7, UGT2B8, and UGT2B11 are 76–91% identical (Table I). In every case the amino-terminal region of the protein between residues 1 and 290 is less homologous to UGT2B17 than the carboxyl-terminal region between residues 291 and 530.

Activity of the UGT2B17 Enzyme—For expression of the UGT2B17 protein, the cDNA was excised into the pBK-CMV vector. To demonstrate the ability of the cDNA to encode a protein the pBK-CMV-UGT2B17 construct was transcribed in vitro using T3 polymerase, and the produced transcript was translated by a rabbit reticulocyte system. Fig. 2 demonstrates the expression of a 53-kDa protein that is similar to other previously characterized UGT2B proteins (6).

HK293 cell homogenate containing stably expressed UGT2B17 was analyzed for aglycone specificity by TLC (Fig. 3). Of the over 60 endogenous and exogenous substances tested for activity, 25 compounds revealed glucuronidation by UGT2B17 (Table II). Glucuronidation of these compounds was absent in control HK293 cell homogenates not containing the exogenous UGT2B17 53-kDa protein. The major endogenous substrates observed were 3α- and 17β-hydroxyandrogens (Fig. 4). Of the androgens tested, testosterone and its 5α-reduced metabolites, DHT, 3α-diol, and ADT, were the best substrates for glucuronidation by UGT2B17. Activities toward etiocholanolone, bile acids, 5α- and 5β-pregnane compounds, and catecholestradiols were also observed; however, the UGT2B17 isoform did not
glucuronidated 3β-hydroxysteroids or estradiol, estrone, and estriol (Table II).

Of the 11 xenobiotics tested, 6 compounds demonstrated reactivity including eugenol, p-nitrophenol, o,o′-biphenyl, p,p′-biphenyl, 1-naphtol, and 4-methylumbelliferone. Eugenol, which is glucuronidated by a majority of the UGT2B isoenzymes, was the best substrate for glucuronidation by UGT2B17. No glucuronidation products were observed when 4-aminophenol, phenolphtalein, imipramine, and 4-OH-flutamide were used as substrates.

To further characterize the specificity of UGT2B17 for the endogenous substrates ADT, DHT, 3α-diol, and testosterone, kinetic analysis was performed using intact HK293 cells stably expressing the enzyme. The Lineweaver-Burk plots in Fig. 5 demonstrate that UGT2B17 has a lower apparent $K_m$ for ADT (0.4 μM) than for the other substrates. The $K_m$ values for DHT, 3α-diol, and testosterone are 0.7, 1.0, and 3.4 μM, respectively (Table III). However, the $V_{max}$ is higher for the active androgen DHT (3.1 pmol/h/μg of DNA) and its precursor testosterone (3.0 pmol/h/μg of DNA) than for the two metabolites ADT (1.3 pmol/h/μg of DNA) and 3α-diol (2.2 pmol/h/μg of DNA). The $V_{max}/K_m$ ratio revealed a similar efficacy of conjugation of these androgens by UGT2B17.

**Tissue Distribution of the UGT2B17 Transcript—**Due to the high homology between members of the UGT2B subfamily, a ribonuclease protection assay was used to measure UGT2B17 transcripts in RNA samples from human tissues (Fig. 6). Using a UGT2B17-specific cRNA probe that differs from the other UGT2B cDNAs, the transcript was detected only in the LNCaP.
The initial screening for reactive substrates was performed in the presence of 6 μM [14C] UDPGA and 94 μM unlabelled UDPGA to optimize sensitivity of the assay. The subsequent determination of glucuronide formation was optimized to measure the glucuronidation activity of UGT2B17 for the various substrates and was performed in the presence of 6 μM [14C] UDPGA and 494 μM unlabelled UDPGA. A reactive substrate – no reactivity, ND (not detectable) indicates that glucuronide formation was not detected under the conditions of the final enzyme activity assay. Activity values presented are the mean ± S.D. of three independent experiments.

| Endogenous compounds | Initial screening | Glucuronide formation (pmol/min/mg protein) |
|----------------------|------------------|---------------------------------------------|
| **C19 STEROIDS**     |                  |                                             |
| Testosterone         | +                | 9                                           |
| Dihydrotestosterone  | +                | 14                                          |
| Androsterone         | +                | 5                                           |
| Epandrosteroi        | –                |                                             |
| Dehydroepiandrosteron| –                |                                             |
| Etiocholanolone       | +                | 13                                          |
| Androst-5-ene-3β, 17β-diol | –            |                                             |
| 5α-Androstane-3α, 17β-diol | +          | 15                                          |
| 5α-Androstane-3α, 17β-diol | –            |                                             |
| 5α-Androstane-3α, 11β, 17β-triol | +      | 8                                           |
| 5α-Androstane-3α, 11α, 17β-triol | + | 5                                           |
| 5β-Androstane-3α, 17β-diol | +          | 14                                          |
| 5β-Androstane-3α, 16α, 17β-triol | + | 11                                          |
| 5β-Androstane-3α, 11α, 17β-triol | + | N.D.                                        |
| **C18 STEROIDS**     |                  |                                             |
| Estrone              | –                |                                             |
| Estradiol            | –                |                                             |
| Estriol              | –                |                                             |
| 1, 3, 5, 10-Estratriene-3, 16α-diol-17-one | + | N.D.                                        |
| 1, 3, 5, 10-Estratriene-2, 3, 17β-triol | +   | N.D.                                        |
| 1, 3, 5, 10-Estratriene-2, 3-diol-17-one | +  | N.D.                                        |
| 1, 3, 5, 10-Estratriene-3, 4, 17β-triol | +  | N.D.                                        |
| 1, 3, 5, 10-Estratriene-2, 3, 16α, 17β-tetrol | + | N.D.                                        |
| 1, 3, 5, 10-Estratriene-3, 4-diol-17-one | +  | N.D.                                        |
| **C21 STEROIDS**     |                  |                                             |
| Progesterone         | –                |                                             |
| 17-OH-Progesterone   | –                |                                             |
| Pregnenolone         | –                |                                             |
| 17-OH-Pregnenolone   | –                |                                             |
| 5α-pregnene-3α, 20-one | +            | N.D.                                        |
| 5α-pregnene-3α, 20α-diol | +     | 8                                           |
| 5α-pregnene-3α, 17α-diol-20-one | –  |                                             |
| 5α-pregnene-3α, 17α-diol-11, 20-one | –  |                                             |
| 5α-pregnene-3α, 17α-diol-20-one | –  |                                             |
| 5β-pregnene-3α, 20α-diol | –  |                                             |
| 5β-pregnene-3α, 17α-diol-20-one | +  | N.D.                                        |
| 5β-pregnene-3α, 6α, 17α-triol-20-one | –  |                                             |
| 11-Deoxycorticol     | –                |                                             |

**DISCUSSION**

In the present study, a novel human cDNA encoding UGT2B17 has been isolated from LNCaP cell and human prostate cDNA libraries. The nucleotide sequence encodes a protein capable of conjugating 3α-hydroxysteroids and 17β-hydroxysteroids such as testosterone, DHT, ADT, and 3α-diol, and the primary structure of UGT2B17 is homologous to steroid-specific UGT enzymes. As found among other members of the UGT2B subfamily, the amino-terminal region from residue 1 to 290 is less homologous with other UGT2B enzymes than the carboxyl-terminal region from residue 291 to 530. Protein chimeric studies have suggested that the highly homologous carboxyl-terminal region contains a domain critical for catalysis as well as a domain responsible for binding UDPGA (34, 47).
UGT2B17 is most homologous to UGT2B15, which is active on 3α-diol and DHT. The two proteins are 93% identical at the amino-terminal region, which has been proposed to contain a domain responsible for determining aglycon specificity. Due to the high homology between the two proteins, it is not surprising that they are both capable of catalyzing the glucuronidation of steroids at the 17β-hydroxyl position; however, UGT2B17 is different from UGT2B15 and can also glucuronidate ADT at the 3α-hydroxyl group. The difference in substrate specificity is most likely conferred by the 21 amino acids that are different between residues 54 and 227 of UGT2B15 and UGT2B17. The other UGT enzyme that is known to glucuronidate the 3α-hydroxyl group of C19 steroids is UGT2B2 isolated from the rat liver; however, its amino-terminal region is only 59% identical with UGT2B17.

**TABLE III**

Kinetic analysis of UGT2B17 stably expressed in HK293 cells

The values of apparent $K_m$ and $V_{max}$ were determined as described under "Experimental Procedures." Results represent the mean of two experiments each performed in triplicate.

| Substrates                  | $K_m$ (app) | $V_{max}$ (pmol/h/μg DNA) | $V_{max}/K_m$ (liters/h/μg DNA⁻¹/μg) |
|-----------------------------|-------------|---------------------------|-------------------------------------|
| Androsterone                | 0.4         | 1.3                       | 325                                 |
| Dihydrotestosterone         | 0.7         | 3.1                       | 440                                 |
| Androstane-3α,17β-diol      | 1.0         | 2.2                       | 220                                 |
| Testosterone                | 3.4         | 3.0                       | 89                                  |

**FIG. 5.** Lineweaver-Burk plots of ADT and DHT (A) and 3α-diol and TESTO (B). Experiments were performed using intact HK293 cells stably expressing UGT2B17. The stable UGT2B17 cells were incubated with the appropriate tritiated substrates ranging from 0.05 to 5 μM for 5 h. Values represent the mean ± S.D. of two experiments each performed in triplicate.

**FIG. 6.** RNase protection analysis of UGT2B17 transcripts. Twenty μg of total RNA isolated from human liver, LNCaP cells, human prostate, and HK293 cells expressing UGT2B15 were hybridized to a specific UGT2B17 cRNA probe. The UGT2B17 probe of 318 bp protected a fragment of 224 bp. The integrity of the RNA was assessed using an 18 S cRNA probe and protected a fragment of 110 bp in each RNA preparation. The sizes of the probe and protected fragments are indicated on the right. All samples were separated on a denaturing 6% polyacrylamide gel.

**FIG. 7.** Tissue distribution of UGT2B17 transcript. Total RNA isolated from human liver, kidney, testis, mammary gland, placenta, adipose, skin, uterus, prostate, lymphoblast, adrenal gland, lung, and LNCaP cells were analyzed by specific RT-PCR analysis as described under "Experimental Procedures." One-fifth of each RT-PCR product was separated on a 1% agarose gel, transferred onto a nylon membrane, and hybridized with the radiolabeled full-length UGT2B17 cDNA probe. The 685-bp PCR product representing UGT2B17 is indicated on the right. The specificity of the RT-PCR product was confirmed by direct sequencing.

The activity of UGT2B17 on the 3α- and 17β-hydroxyl groups of androgens is consistent with our previous observations of glucuronidation of testosterone, DHT, 3α-diol, and ADT in LNCaP cells. The specificity of the RT-PCR product was confirmed by direct sequencing.
tion at the two positions. As previously found in LNCaP cells where 11-hydroxy C19 steroids were poor substrates for glucuronidation, the UGT2B17 enzyme expressed in HK293 cells is also less active on steroid substrates (5α-androstane-3α, 11β,17β-triol, and 5α-androstane-3α,11α,17β-triol) that contain a hydroxyl group at position 11. It is possible that a hydroxyl group at position 11 of the steroid molecule interferes with the proper interaction with the aglycon binding domain at the amino-terminal region of the UGT protein. In addition, the observation that UGT2B17 is active on ADT but is not active on the 3β-hydroxy group of epiaandrosterone demonstrates the stereospecificity of the enzyme. Similarly to other UGT2B enzymes isolated to date, UGT2B17 is highly reactive toward eugenol in addition to other endogenous and exogenous substrates. It is clear that members of the UGT2B subfamily express overlapping substrate specificities; however, UGT2B17 is presently the only human enzyme capable of glucuronidating C19 steroids at the 3α-hydroxyl group, where the apparent K_m for ADT is 0.4 μM. In intact HK293 cells stably expressing UGT2B17, the affinities of the enzyme for ADT, DHT, 3α-diol, and testosterone are similar, as determined by their K_m values. As well, determination of the V_max/K_m ratio indicates a similar relative enzyme efficiency for the substrates. Although it is difficult to compare apparent K_m values obtained by different methods, it is interesting to note that the K_m value of 1.0 μM for 3α-diol and 0.7 μM for DHT with the UGT2B17 enzyme in intact cells is approximately 10-fold lower than the reported K_m values of these substrates with the UGT2B15 enzyme in cell homogenates. The apparent K_m of UGT2B15 for 3α-diol as determined in stably transfected intact HK293 cells was 2.0 μM (data not shown) as compared with a K_m value of 15 μM as determined in cell extracts (36). Determination of K_m values using intact cells and cell homogenates both have the intrinsic limitation of potential interference with other cellular components. However, incubation of HK293 cells with ADT, DHT, 3α-diol, and testosterone did not lead to conversion into other products as demonstrated by HPLC (data not shown), and these substrates diffuse freely into the cells and therefore have equal access to the UGT2B enzymes in the endoplasmic reticulum.

RNase protection analysis clearly demonstrates the expression of UGT2B17 in LNCaP cells, which is a model of the human prostate. Although this assay did not detect expression of UGT2B17 in an RNA sample from a human prostate, it was surprising that expression was also not detected in the liver. When similar experiments were performed with a probe specific for UGT2B15, expression was detected in the liver and not in the prostate, therefore demonstrating the higher level of expression in the liver (8). The nondetection of UGT2B17 by RNase protection in both the liver and prostate indicates a lower level of expression in both tissues and shows that the liver does not necessarily express larger amounts of this enzyme as is the case for UGT2B15. The smaller protected bands in Fig. 5 seen with the RNA sample from liver are consistent with this tissue expressing other homologous UGT2B transcripts.

The expression of UGT2B17 in several human tissues was demonstrated by RT-PCR followed by Southern blot analysis. Compared with the expression of UGT2B15 transcript in the human liver, the expression of UGT2B17 is relatively low in the human liver and prostate. It is possible that UGT2B17 protein has a low turnover, thus not necessitating high levels of transcript to sustain glucuronidation activity. Initial experiments in LNCaP cells indicate that androgen-specific UGT enzymes are stable where 60% of glucuronidation activity is retained following the incubation of cells with 25 μg/ml of cycloheximide for 24 h (48).

Previously, glucuronidation of androgens in human extrahepatic tissues such as the prostate was examined but yielded conflicting results due to the extremely low level of steroid UGT activity in the prostate as compared with the liver (49). However, the measurement of UGT activities in these studies may be difficult to interpret due to the labile nature of human UGT enzymes during preparation of tissue samples (4). The demonstration of UGT2B15 and UGT2B17 expression in the human prostate as well as significant levels of ADT-G and 3α-diol-G detected in this tissue clearly indicate the potential importance of glucuronidation in peripheral tissues, which may also contribute to the level of circulating glucuronidated steroids found in humans. It has been demonstrated that ADT-G exceeds by 5–10-fold the level of 3α-diol-G in the plasma, follicular fluid, and prostate, indicating that ADT-G represents the predominant final product of androgen metabolism in steroid target tissues. In men, the constant ratio of 7-fold (p < 0.0001) between the levels of ADT-G and 3α-diol-G in the plasma also suggests that UGT2B17 is responsible for conjugating both ADT and 3α-diol (Fig. 8). It must be mentioned that UGT2B17 only glucuronidates the hydroxyl group at position 17 of 3α-diol (data not shown) and that, in the circulation, only 3α-diol glucuronidated at position 17 was reported to be present (50). The high level of ADT-G in the circulation may be explained by the higher formation of its substrate ADT, and in fact, 17β-hydroxysteroid dehydrogenase type II, which is present in tissues such as the prostate, favors ADT formation over 3α-diol (51).

In summary, the present study clearly shows that several human extrahepatic tissues express UGT2B17, which may contribute to plasma levels of ADT-G and 3α-diol-G. Our findings also concur with the concept that the glucuronidation of androgens by specific UGT2B enzymes in the human prostate may be a means by which the level of active androgens are regulated in this tissue.

Acknowledgments—We thank Dr. Pei Min Rong for technical assistance in DNA sequencing and Bernard Lavalle for statistical analysis. We thank Dr. Thomas R. Tephly and Mitchell D. Green for helpful discussions and for providing the HK293 cell line expressing UGT2B15 cDNA.

REFERENCES

1. Dutton, G. J. (1980) Glucuronidation of Drugs and Other Compounds, CRC Press, Inc., Boca Raton, FL
Androgen-specific UDP-Glucuronosyltransferase
Isolation and Characterization of a Novel cDNA Encoding a Human UDP-Glucuronosyltransferase Active on C_{19} Steroids
Martin Beaulieu, Eric Lévesque, Dean W. Hum and Alain Bélanger

J. Biol. Chem. 1996, 271:22855-22862.
doi: 10.1074/jbc.271.37.22855

Access the most updated version of this article at http://www.jbc.org/content/271/37/22855

Alerts:
• When this article is cited
• When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 48 references, 9 of which can be accessed free at http://www.jbc.org/content/271/37/22855.full.html#ref-list-1