0090-9556/98/2608-0795–801$02.00/0
DRUG METABOLISM AND DISPOSITION
Vol. 26, No. 8
Printed in U.S.A.

DIFFERENTIAL REGULATION OF INDIVIDUAL SULFOTRANSFERASE ISOFORMS BY PHENOBARBITAL IN MALE RAT LIVER

MELISSA RUNGE-MORRIS, KELLY ROSE, CHARLES N. FALANY, AND THOMAS A. KOCAREK

Institute of Chemical Toxicology, Wayne State University (M.R.-M., K.R., T.A.K.), and Department of Pharmacology and Toxicology, University of Alabama at Birmingham (C.N.F.)

(Received January 22, 1998; accepted April 1, 1998)

This paper is available online at http://www.dmd.org

ABSTRACT:

Xenobiotics that induce the cytochromes P450 also produce changes in rat hepatic sulfotransferase (SULT) gene expression. In the present study, male Sprague-Dawley rats were treated for 3 consecutive days with doses of phenobarbital (PB) that induce cytochrome P450 2B1/2 expression. The effects of PB treatment on hepatic aryl SULT (SULT1) and hydroxysteroid SULT (SULT2) mRNA and immunoreactive protein levels and on mRNA expression of individual SULT1 and SULT2 enzyme isoforms were characterized. PB suppressed SULT1A1 mRNA levels, increased the expression of the SULT-Dopa/tyrosine isoform, and did not produce significant changes in SULT1C1 and SULT1E2 mRNA expression. In rats injected with the highest test dose of PB (100 mg/kg), hepatic SULT1A1 mRNA levels were decreased to ~42% of control levels and SULT-Dopa/tyrosine mRNA levels were increased to ~417% of vehicle-treated control levels. Like the SULT1 subfamily, individual members of the SULT2 gene subfamily were differentially affected by PB treatment. PB (35, 80, and 100 mg/kg) suppressed SULT20/21 mRNA expression to ~61, ~30, and ~41% of vehicle-treated control levels, respectively. In contrast, SULT60 mRNA levels were increased to ~162% of control levels and SULT40/41 mRNA levels were increased to ~416% of vehicle-treated control levels in rats treated with 100 mg/kg PB. These studies support a complex role for PB-mediated effects on the SULT multigene family in rat liver. Because individual SULT1 and SULT2 enzyme isoforms are known to metabolize a variety of potentially toxic substrates, varied responses to PB among members of the SULT multigene family might have important implications for xenobiotic hepatotoxicity.

Maintaining control of hepatic drug-metabolizing enzyme gene expression is essential to the multifaceted process of xenobiotic detoxication. In rat liver, members of the SULT1[1,2] gene subfamily catalyze the sulfation (or *o*-sulfonation) of a number of important endogenous and exogenous substrates, ranging from phenols to biogenic amines (Duffel *et al.,* 1991). The SULT2 gene subfamily catalyzes the sulfation of aliphatic alcohols, hydroxysteroids, bile acids, and other conceivably toxic substrates (Jakoby *et al.,* 1980). In metabolically active tissues such as the liver, enzymatic sulfation of xenobiotics may culminate in the excretion of more polar end-products (detoxication). Alternatively, unbridled generation of highly reactive sulfate esters in toxicant target tissues may flood the system with electrophilic species that readily form covalent adducts with critical cellular nucleophiles, such as DNA, RNA, and protein (toxicant bioactivation). In rat liver, the ability of the SULT1 and SULT2 enzymes to metabolize xenobiotics has been suggested to play a key role in hepatocarcinogenesis. For example, SULT2 enzymes bioactivate hydroxymethyl polycyclic aromatic hydrocarbon procarcinogens, such as 5-hydroxymethylchrysene and 7,12-dihydroxymethylbenz[*a*]anthracene, to reactive electrophilic intermediates (Okuda *et al.,* 1989; Ogura *et al.,* 1990b), and SULT1-mediated bioactivation is thought to amplify the carcinogenicity of *N*-hydroxy-2-acetylaminofluorene (Yerokun *et al.,* 1992; Meerman *et al.,* 1981).

Members of the SULT1 and SULT2 gene subfamilies represent the principal SULT enzymes that are essential to rat hepatic xenobiotic metabolism. There are at least four SULT1 enzyme isoforms and three individual SULT2 enzyme isoforms present in rat liver (Sakakibara *et al.,* 1995; Yamazoe *et al.,* 1994; Liu *et al.,* 1996). Historically, the SULT1 and SULT2 enzymes have not been classified as "xenobiotic-inducible" enzymes. However, we previously demonstrated that SULT gene expression is affected by aryl hydrocarbon receptor agonist, P450-inducing agents. For example, *in vivo* treatment with the CYP1A1 inducer 3-methylcholanthrene suppressed rat hepatic SULT2 (HST-a) mRNA expression in a dose-dependent manner (Runge-Morris and Wilusz, 1994). Similarly, we and others have shown that *in vivo* treatment with CYP3A-inducing doses of xenobiotic steroidal chemicals, such as dexamethasone or pregnenolone-16α-carbonitrile, increases rat hepatic SULT1 and SULT2 mRNA expression (Runge-Morris *et al.,* 1996; Liu and Klaassen, 1996a).

The effects on SULT gene expression of another major class of xenobiotic P450-inducing agents, namely PB and "PB-like" inducers, have not been systematically established. PB and the PB-like agents,

This work was supported by National Institutes of Health Grants ES05823 (M.R.-M.) and HL50710 (T.A.K.).

[1] Abbreviations used are: SULT1, aryl sulfotransferase; SULT, sulfotransferase; ANOVA, analysis of variance; CYP or P450, cytochrome P450; PB, phenobarbital; SDS, sodium dodecyl sulfate; SULT2, hydroxysteroid sulfotransferase; ECL, enhanced chemiluminescence; EST, estrogen sulfotransferase; RT-PCR, reverse transcription-polymerase chain reaction; SSC, standard saline citrate.

[2] Proposed nomenclature for the SULT gene family is from the Workshop on Sulfotransferase Enzyme Nomenclature, Annual Meeting of the International Society for the Study of Xenobiotics (Seattle, WA, August 28, 1995).

Send reprint requests to: Melissa Runge-Morris, M.D., Institute of Chemical Toxicology, Wayne State University, 2727 Second Avenue, Detroit, MI 48201.

TABLE 1

SULT isoform-specific oligonucleotide probes

Probe	References	GenBank Accession No.	Sequence (5′ to 3′)	Nucleotide Positions	Hybridization Temperature
SULT1A1	Ozawa *et al.*, 1990; Yerokun *et al.*, 1992	X52883	CTTCACATGCACTAGCGGTG	82–101	50°C
SULT1C1	Nagata *et al.*, 1993	L22339	CACCTAGTGTGGAAGGTCTG	1050–1069	40°C
SULT1E2	Demyan *et al.*, 1992	M86758	GCACTCCAGGTCAGGTATTC	364–383	45°C
SULT-Dopa/tyrosine	Sakakibara *et al.*, 1995	U38419	TCCCAACCCAGTGCAAAAGC	157–176	55°C
SULT20/21[a]	Ogura *et al.*, 1989	M31363, D14987, D14988	CCTTTCCTCATGAGGCCAGT	761–780	50°C
SULT40/41[b]	Ogura *et al.*, 1990a	M33329, X63410	TGTCTAATTCTCGCATAGAC	547–566	50°C
SULT60	Watabe *et al.*, 1994; Liu *et al.*, 1996	D14989	TTTCTTCTCCAGGGCGATCT	436–455	45°C

[a] The SULT20/21 oligonucleotide probe corresponds to the indicated nucleotide sequence in SULT20 (GenBank accession number M31363).
[b] The SULT40/41 oligonucleotide probe corresponds to the indicated nucleotide sequence in SULT40 (GenBank accession number M33329).

such as certain organochlorine compounds, polychlorinated biphenyls, and imidazole antimycotic agents, induce CYP2B1/2 gene expression and cause pleiotropic effects on hepatic gene expression that include increases in P450, glutathione-*S*-transferase, epoxide hydrolase, and UDP-glucuronosyltransferase enzyme expression (Lubet *et al.*, 1992). In addition, PB may produce divergent effects on closely related drug-metabolizing enzyme isoforms. Preliminary data in our laboratory suggested that *in vivo* treatment of rats with the archetypical CYP2B1/2 inducer PB produces alterations in hepatic SULT1 and SULT2 gene expression (Runge-Morris and Vento, 1994). In this report, the effects of PB on SULT1 and SULT2 gene subfamily expression and on SULT1/2 isoform-specific mRNA expression in rat liver were characterized.

Materials and Methods

Materials. PB and molecular biology-grade reagents were purchased from Sigma Chemical Co. (St. Louis, MO). Trizol reagent and Klenow fragment were obtained from GIBCO BRL (Grand Island, NY). Terminal deoxynucleotidyl transferase was purchased from Promega Corp. (Madison, WI). SDS-polyacrylamide gel electrophoresis reagents were obtained from Bio-Rad Laboratories (Hercules, CA). ECL Western blotting kits and [α-^{32}P]dATP (>3000 Ci/mmol) were purchased from Amersham (Arlington Heights, IL). Nylon hybridization membranes (Genescreen Plus) and [α-^{32}P]dATP (>6000 Ci/mmol) were purchased from Du Pont NEN Co. (Boston, MA).

Animals. Adult male Sprague-Dawley rats (age, ~55 days; 264–280 g) were purchased from Harlan, Inc. (Indianapolis, IN). Rats were housed in steel mesh cages and were supplied with Purina Rodent Chow (Purina, Indianapolis, IN) and water *ad libitum* before the initiation of experimental protocols.

***In Vivo* Drug Treatment.** Based on preliminary dose-optimization studies, rats were treated for 3 consecutive days with ip injections of control 0.9% saline solution or PB (35, 80, or 100 mg/kg in water). The doses of PB used in this study did not produce signs of clinical toxicity in treated animals. After the final drug treatment, rats were fasted for 24 hr and then killed with lethal pentobarbital injections (120 mg/kg, ip). The livers were then perfused *in situ* with ice-cold 0.9% saline solution and were powdered under liquid nitrogen. To permit statistical analysis of the data, three rats were included in each experimental group ($N = 3$).

Origin of cDNA and Oligonucleotide Probes. Rat SULT1 (ASTIV) and SULT2 (HST-a) cDNA probes were generated using RT-PCR, as described previously (Runge-Morris *et al.*, 1996; Runge-Morris and Vento, 1995). The 799-base pair SULT1 cDNA probe was prepared by RT-PCR using primers corresponding to nucleotides 97–112 and 881–896 of the previously published rat hepatic SULT1 cDNA sequence (GenBank accession number X52883) (Yerokun *et al.*, 1992; Ozawa *et al.*, 1990). The 518-base pair SULT2 cDNA probe was prepared by RT-PCR using oligonucleotide primers corresponding to nucleotides 306–321 and 809–824 of the ST-20 SULT2 cDNA sequence (GenBank accession number M31363) (Ogura *et al.*, 1989). The SULT-Dopa/tyrosine oligonucleotide probe used in these studies was selected using Oligo PrimerAnalysis software (National Biosciences Inc., Plymouth, MN) and published cDNA sequence information for rat hepatic SULT-dopa/tyrosine (GenBank accession number U38419) (Sakakibara *et al.*, 1995) (table 1). Oligonucleotide probes specific for the SULT-Dopa/tyrosine isoform and for SULT1 isoforms (SULT1A1, SULT1C1, and SULT1E2) and SULT2 isoforms (SULT20/21, SULT40/41, and SULT60) previously described and characterized by Liu *et al.* (1996) were synthesized by National Biosciences Inc. Specific sequence and hybridization information on the isoform-specific oligonucleotide probes used in these studies is provided in table 1.

Northern Blot Analyses. Total RNA was prepared from 1-g samples of powdered liver as previously described (Puissant and Houdebine, 1990; Runge-Morris *et al.*, 1996; Runge-Morris and Vento, 1995). Poly(A)$^+$ RNA was prepared from 1-mg samples of total RNA using the Qiagen digotex mRNA Midi kit (Chatsworth, CA), in accordance with the manufacturer's instructions, and poly(A)$^+$ RNA (2 μg/lane) was analyzed on Northern blots hybridized with isoform-specific SULT oligonucleotide probes. Briefly, Northern blots were prehybridized overnight in a prehybridization/hybridization solution containing 5× Denhardt's solution (0.1% Ficoll, 0.1% polyvinylpyrrolidone, 0.1% bovine serum albumin), 6× SSC (1× SSC contains 150 mM sodium chloride and 15 mM sodium citrate), 1% SDS, 200 μg/ml sheared, hydrolyzed, salmon sperm DNA, and 200 μg/ml polyadenylic acid and were hybridized overnight at the temperature of dissociation minus 4°C–21°C, in prehybridization/hybridization solution containing 20×10^6 cpm/ml levels of individual SULT1 or SULT2 oligonucleotide probes labeled using terminal deoxynucleotidyl transferase (Collins and Hunsaker, 1985). After hybridization, filters hybridized with SULT1E2, SULT1C1, SULT-Dopa/tyrosine, or SULT2 oligonucleotide probes were washed twice at hybridization temperature for 1 hr with 5× SSC/0.5% SDS and once for 1 hr with 5× SSC/0.1% SDS and were exposed to autoradiographic film. Filters hybridized with the SULT1A1 oligonucleotide probe were washed as described above but also received a more stringent wash with 2× SSC/0.1% SDS at the temperature of dissociation minus 9°C for 1 hr. Alternatively, filters were hybridized with labeled SULT1 or SULT2 cDNA probes, washed, and exposed to autoradiographic film as previously described (Runge-Morris *et al.*, 1996). To normalize for RNA loading and transfer, blots were stripped of labeled SULT probes as previously described (Runge-Morris *et al.*, 1996) and were rehybridized with ^{32}P-labeled β-actin cDNA probe (prepared using random-prime labeling).

Western Blot Analyses of Immunoreactive SULT1 and SULT2 Protein Levels in Rat Liver Cytosol. Polyclonal antiserum to rat SULT1 protein was prepared and characterized as described previously (Hirshey *et al.*, 1992; Hirshey and Falany, 1990). Polyclonal antiserum to rat SULT2 (formerly called HST-a) protein was prepared as described previously (Runge-Morris and Wilusz, 1994). Rat hepatic cytosol was prepared from the livers of rats treated *in vivo* with vehicle or PB, and protein concentrations in cytosol samples were determined according to established methods (Smith *et al.*, 1985). Hepatic cytosol samples (3.0 μg/lane for anti-SULT1 antiserum blots, 7.5 μg/lane for anti-SULT2 antiserum blots) were fractionated by SDS-polyacrylamide gel electrophoresis (Laemmli, 1970), and immunoreactive SULT1 and SULT2 protein levels were determined using ECL Western blot analysis, as described previously (Runge-Morris and Wilusz, 1994; Runge-Morris and Vento, 1995).

Statistical Analysis of the Data. Northern blot autoradiographs and Western blot chemiluminescence films were analyzed by scanning laser densitometry using the ImageQuant software package (Molecular Dynamics, Sunnyvale, CA), as described previously (Runge-Morris *et al.*, 1996). Statistical analysis of Northern blot data was performed using one-way ANOVA, followed by Dunnett's test to compare treatment effects with control. The

dose-response data were also analyzed using linear regression, to assess whether the slopes of the best-fit lines were significantly different from 0. Western blot data were analyzed using unpaired *t* tests. All statistical tests were performed using the Prism software package (GraphPad, San Diego, CA).

Results

The effects of *in vivo* treatment with PB on the mRNA and protein expression of the rat hepatic SULT1 and SULT2 gene subfamilies and on the mRNA expression of individual SULT1 and SULT isoforms were investigated. Preliminary studies in our laboratory suggested that the PB-mediated effects on SULT1 and SULT2 gene expression might be subtle. However, because of the potential for Northern blotting with SULT cDNA probes to mask more demonstrable PB-mediated effects on single SULT isoforms, we determined to assess the effects of PB treatment on the mRNA expression of specific SULT1 and SULT2 isoforms. Gender-dependent differences in rat hepatic SULT1 and SULT2 gene expression (Song *et al.*, 1990; Runge-Morris and Wilusz, 1991; Runge-Morris, 1994; Singer and Sylvester, 1976) and regulation (Ueda *et al.*, 1997; Liu and Klaassen, 1996a,b,c) have been described. The present study focused on characterizing PB-mediated effects on hepatic SULT1 and SULT2 expression in male rats because we found detectable mRNA levels for individual SULT enzyme isoforms in the livers of mature male rats and because our most recent mechanistic studies on the regulation of hepatic SULT1 and SULT2 gene expression by glucocorticoid-class P450 inducers were performed with mature male rats (Runge-Morris *et al.*, 1996).

Northern blots of poly(A)$^+$ RNA isolated from the livers of rats treated with PB or saline vehicle were hybridized with a SULT1 cDNA probe that was originally designed to maximize the detection of SULT1A1 and related SULT1 isoforms, as described in *Materials and Methods*. This experiment revealed that the lowest test dose of PB (35 mg/kg) produced a moderate, but statistically insignificant (by ANOVA and Dunnett's test), increase in rat hepatic SULT1 mRNA levels, to ~138% of vehicle-treated control levels (fig. 1). Higher doses of PB failed to produce substantial alterations in SULT1 mRNA levels, in comparison with vehicle-treated control (fig. 1).

To complement statistical analysis of the data by ANOVA and Dunnett's test, linear regression analyses of the dose-response data were performed, as a simple method to determine the degree to which PB treatments produced dose-dependent increases or decreases in SULT mRNA expression. If linear regression analysis supported a dose-dependent change in SULT mRNA expression in response to PB treatment, the slope of the linear regression best-fit line was significantly different from 0 (indicated as * in figs.). Treatment for 3 consecutive days with increasing doses of PB (35, 80, or 100 mg/kg) tended to suppress rat hepatic SULT2 mRNA expression in a relatively dose-dependent manner, to levels that were ~88, ~62, and ~58% of control levels, respectively (fig. 1), and the slope of the best-fit line approximating this dose-response relationship was significantly less than 0. As expected, PB-treated rats displayed significant increases in the amounts of hepatic CYP2B1/2 mRNA, with maximal CYP2B1/2 induction occurring at a PB dose of 80 mg/kg and being sustained at the 100 mg/kg dose (data not shown).

The effects of PB on immunoreactive SULT1 and SULT2 protein levels were also evaluated, and results were compared with PB-mediated changes in SULT1 and SULT2 mRNA levels. Western blots were performed with rat hepatic cytosolic protein isolated from rats treated with PB or saline vehicle. In the absence of available isoform-specific anti-SULT antibodies, class-specific anti-SULT1 and -SULT2 antisera were used in ECL Western blot analyses. The polyclonal anti-SULT1 antiserum used in these studies was designed

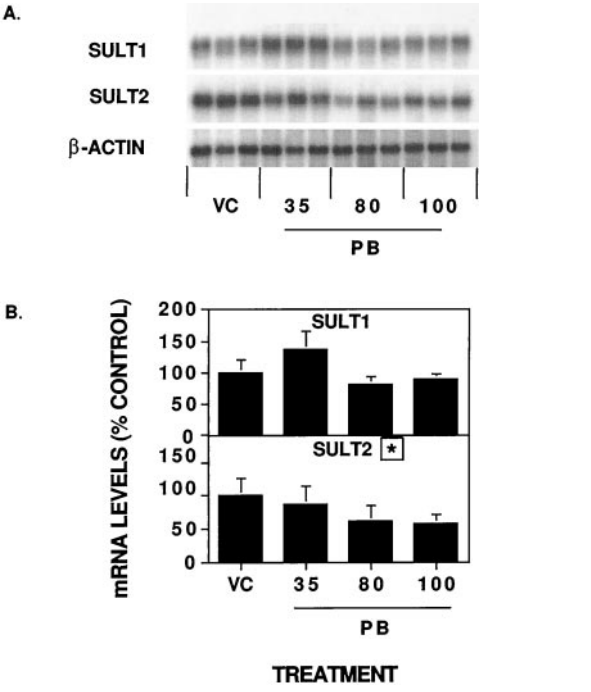

FIG. 1. *Effects of* in vivo *treatments with PB on SULT1 and SULT2 mRNA levels in male rat liver.*

Adult male Sprague-Dawley rats (three rats/treatment group) were treated for 3 consecutive days with saline vehicle (*VC*) or PB (35, 80, or 100 mg/kg, ip) and were sacrificed 24 hr after the last treatment. Poly(A)$^+$ RNA was prepared from a sample of each individual liver, and aliquots (2 μg/lane) were analyzed by Northern blot hybridization, using a cDNA probe to SULT1 or SULT2. Hybridizable RNA bands were detected by autoradiography, and band intensities were estimated by scanning laser densitometry. After densitometric analysis, SULT probes were washed from the blots, and the blots were rehybridized with a β-actin cDNA probe. *A*, autoradiographs of Northern blots hybridized with SULT1 or SULT2 cDNA. A representative autoradiograph of a blot rehybridized with β-actin cDNA is also shown. *B*, graphical representations of SULT1 and SULT2 mRNA band intensities. The SULT1 and SULT2 band intensities were normalized to the respective β-actin band intensities and are presented as percentages of control (mean $\pm$ SD, $N = 3$). * The slope of the best-fit line for the dose-response data is significantly different from 0 ($p < 0.05$).

to detect rat hepatic "minoxidil SULT," as described previously (Hirshey *et al.*, 1992; Hirshey and Falany, 1990), and is expected to cross-react with SULT1A1 subunit protein and related isoform subunits. The anti-SULT2 antiserum used in these studies was prepared as described previously (Runge-Morris *et al.*, 1996; Runge-Morris and Wilusz, 1994). Given the strong structural sequence similarities among SULT2 subunit proteins, it is likely that our polyclonal anti-SULT2 antiserum detects all of the known SULT2 isoforms, including SULT20/21, SULT40/41, and SULT60 (Watabe *et al.*, 1994).

In contrast to the SULT2 Northern blot data, which indicated a progressive decline in SULT2 mRNA expression with increasing PB doses, the most demonstrable suppression of immunoreactive SULT2 protein expression occurred at the lowest test dose of PB (SULT2 protein was suppressed to levels that were ~43% of vehicle-treated control levels), an effect that approached statistical significance ($p = 0.054$). In accordance with the SULT1 mRNA data, which showed minimal changes in hepatic SULT1 mRNA levels in response to PB, amounts of hepatic immunoreactive SULT1 protein were not significantly altered in PB-treated rats (fig. 2).

A recognized limitation of using SULT1 and SULT2 class-specific cDNA probes for Northern blots and polyclonal antisera for Western blots is that these tools lack adequate specificity to detect PB-mediated effects on individual SULT isoforms. Therefore, the impact of

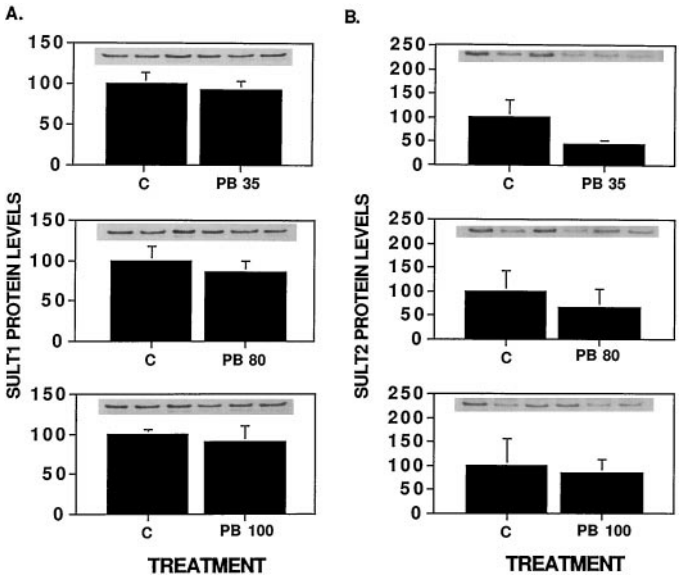

FIG. 2. *Effects of* in vivo *treatments with PB on immunoreactive SULT1 and SULT2 protein levels in male rat liver.*

Rats were treated as described in the legend to fig. 1. Cytosol was prepared from a sample of each individual liver, and aliquots (3 μg of protein/lane for SULT1 and 7.5 μg of protein/lane for SULT2) were analyzed in Western blots for amounts of immunoreactive SULT1 (*A*) or SULT2 (*B*) protein. Each Western blot contained three control liver cytosol samples (*C*) and three liver cytosol samples prepared from rats treated with one dose of PB (35, 80, or 100 mg/kg). Immunoreactive proteins were detected by enhanced chemiluminescence, and band intensities were estimated by scanning laser densitometry. The immunoreactive SULT1 and SULT2 bands were of the expected sizes (~35 and ~30 kDa, respectively). The semiquantitative densitometric data are presented as percentages of control (mean ± SD, $N = 3$).

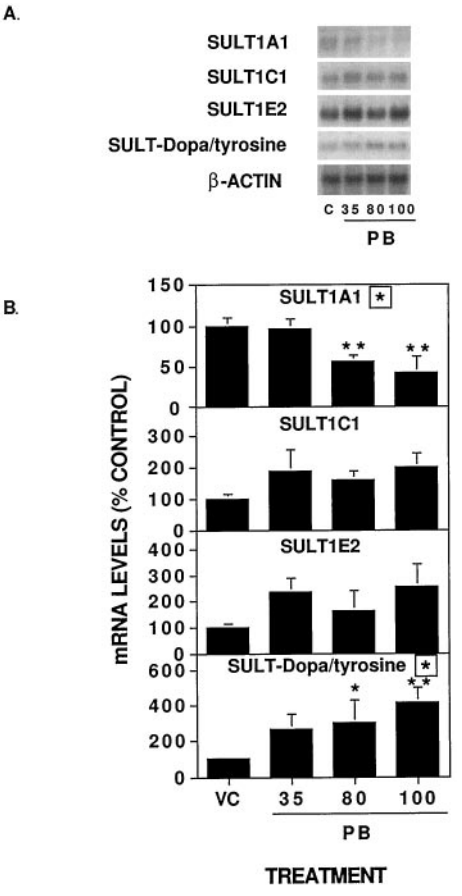

FIG. 3. *Effects of* in vivo *treatments with PB on individual SULT1 isoform mRNA levels in male rat liver.*

Rats were treated and samples of poly(A)$^+$ RNA were prepared as described in the legend to fig. 1. *A*, autoradiographs of Northern blots hybridized with SULT1 isoform-specific oligonucleotide probes. Aliquots of the poly(A)$^+$ RNA samples prepared from the three livers comprising each treatment group were pooled, and 2 μg of the pooled samples were analyzed by Northern blot hybridization, using oligonucleotide probes directed against SULT1A1, SULT1C1, SULT1E2, or SULT-Dopa/tyrosine mRNA. A representative autoradiograph of a blot rehybridized with β-actin cDNA is also shown. *B*, graphical representations of SULT1 isoform mRNA band intensities. As described in the legend to fig. 1, aliquots of poly(A)$^+$ RNA (2 μg/lane), prepared from samples of each individual liver, were analyzed by Northern blot hybridization, using isoform-specific SULT1 oligonucleotide probes. Hybridizable RNA band intensities were estimated by scanning laser densitometry. SULT1 isoform mRNA band intensities were normalized to the respective β-actin band intensities and are presented as percentages of control (mean ± SD, $N = 3$). *, significantly different from control ($p < 0.05$); **, significantly different from control ($p < 0.01$). ⊠ The slope of the best-fit line for the dose-response data is significantly different from 0 ($p < 0.05$).

PB treatment on the mRNA expression of individual rat hepatic SULT1 and SULT2 isoforms was investigated using isoform-specific oligonucleotide probes. Of the SULT1 isoforms examined, SULT1A1 was the only isoform that showed a significant dose-dependent suppressive response to PB treatment. At PB doses of 80 and 100 mg/kg, SULT1A1 mRNA levels declined significantly, to ~57 and ~42% of control levels, respectively (fig. 3). In contrast, moderate increases in SULT1C1 and SULT1E2 mRNA levels were observed in PB-treated rats, although these changes were not statistically significant (fig. 3). The SULT-Dopa/tyrosine isoform was significantly induced, in a dose-dependent manner. In rats treated with 100 mg/kg PB, hepatic SULT-Dopa/tyrosine mRNA levels were increased significantly (to ~417% of control levels) (fig. 3).

Although the SULT2 isoforms are closely related structurally and functionally (Watabe *et al.*, 1994), PB produced markedly different effects on the expression of individual SULT2 isoforms (fig. 4). When mRNA samples from similar treatment groups were pooled, increasing doses of PB clearly produced suppression of SULT20/21, induction of SULT40/41, and a lesser increase in the expression of SULT60 (fig. 4*A*). Triplicate analysis of separate poly(A)$^+$ RNA samples demonstrated dose-dependent decreases in SULT20/21 mRNA expression in response to increasing doses of PB (35, 80, and 100 mg/kg), which were statistically significant by linear regression analysis (to levels that were ~61, ~30, and ~41% of vehicle-treated control levels, respectively) (fig. 4*B*). In direct contrast, these same doses of PB produced significant (by linear regression) dose-dependent increases in the amounts of hepatic SULT40/41 mRNA (to ~261, ~313, and ~416% of control levels, respectively) and SULT60 mRNA (to ~97, ~133, and ~162% of control levels, respectively) (fig. 4*B*).

Discussion

Classes of hormones and xenobiotic agents that induce P450 expression also affect the expression of the SULT multigene family. We previously reported that SULT2 mRNA expression in female rat liver is markedly suppressed in response to *in vivo* treatment with the CYP1A1-inducing agent 3-methylcholanthrene (Runge-Morris and Wilusz, 1994). Similarly, prototypical glucocorticoid/steroid chemical inducers of CYP3A induce the mRNA expression of both the SULT1 and SULT2 gene subfamilies *in vivo* and in primary rat hepatocyte cultures (Liu and Klaassen, 1996a; Runge-Morris *et al.*, 1996). The effects of P450 inducers on SULT gene expression appear to be complex. *In vivo* treatment of male and female rats with steroidal chemicals produces different patterns of effects on the mRNA expression of individual SULT isoforms in rat liver (Liu and Klaassen,

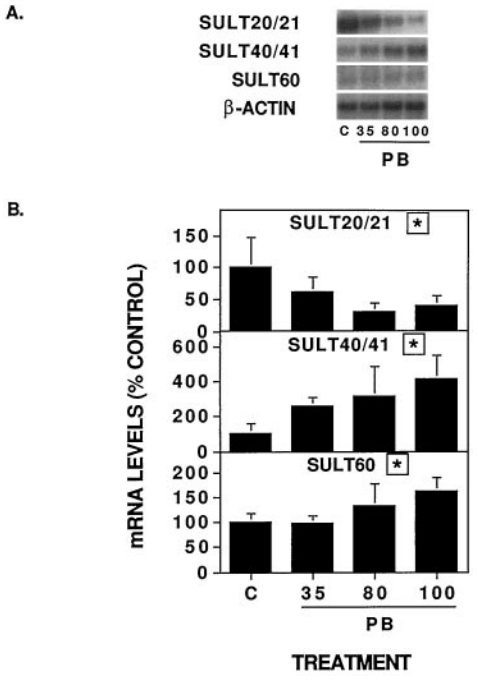

FIG. 4. *Effects of* in vivo *treatments with PB on individual SULT2 isoform mRNA levels in male rat liver.*

Rats were treated and samples of poly(A)$^+$ RNA were prepared as described in the legend to fig. 1. *A*, autoradiographs of Northern blots hybridized with SULT2 isoform-specific oligonucleotide probes. Aliquots of the poly(A)$^+$ RNA samples prepared from the three livers comprising each treatment group were pooled, and 2 μg of the pooled samples were analyzed by Northern blot hybridization, using oligonucleotide probes directed against SULT20/21, SULT40/41, or SULT60 mRNA. A representative autoradiograph of a blot rehybridized with β-actin cDNA is also shown. *B*, graphical representations of SULT2 mRNA band intensities. As described in the legend to fig. 1, aliquots of poly(A)$^+$ RNA (2 μg/lane), prepared from samples of each individual liver, were analyzed by Northern blot hybridization, using isoform-specific SULT2 oligonucleotide probes. Hybridizable RNA band intensities were estimated by scanning laser densitometry. SULT2 isoform mRNA band intensities were normalized to the respective β-actin band intensities and are presented as percentages of control (mean $\pm$ SD, $N = 3$). * The slope of the best-fit line for the dose-response data is significantly different from 0 ($p < 0.05$).

1996a). In this study, we have demonstrated that treatment with the prototypical CYP2B1/2-inducing agent PB also produces differential effects on individual SULT1 and SULT2 isoforms in rat liver.

Isoform-specific effects on drug-metabolizing enzyme gene regulation have the potential to shift the balance of drug metabolism and xenobiotic detoxication profiles in the liver. Despite the considerable substrate-specificity overlap among members of each SULT gene subfamily, isoform-specific differences in SULT substrate preferences have been reported, particularly within the heterogeneous SULT1 gene subfamily. For example, SULT1A1 readily catalyzes the sulfation of minoxidil and a variety of phenols but displays lesser sulfating activity toward the carcinogenic substrate *N*-hydroxy-2-acetylaminofluorene (Nagata *et al.*, 1993; Yamazoe *et al.*, 1994). In contrast, SULT1C1 shares ~50% amino acid sequence identity with SULT1A1 (Yamazoe *et al.*, 1994) and exhibits robust sulfating activity toward this carcinogenic intermediate (Nagata *et al.*, 1993; Yamazoe *et al.*, 1994). Therefore, xenobiotic agents that preferentially induce or suppress rat hepatic SULT1C1 gene expression may alter the course of hepatocarcinogenesis by interfering with intrahepatic bioactivation or detoxication of this procarcinogen.

EST (SULT1E2) (Demyan *et al.*, 1992) catalyzes the sulfation of estrogenic steroids. However, there appears to be multiplicity within the EST subclass of SULT1 enzymes. To date, five EST cDNA sequences have been published; they appear to represent two distinct isoforms. Comparative sequence analysis of the deduced amino acid sequences for these two closely related isoforms indicates that they share approximately 94% sequence identity (Falany *et al.*, 1995). The SULT1E2 oligonucleotide probe used in the present study was designed to detect the EST isoform described by Demyan *et al.* (1992) (GenBank accession number M86758) and is a perfect match to the rEST-3 (Falany *et al.*, 1995) (GenBank accession number S76489) and STe1 (Rikke and Roy, 1996) (GenBank accession number U50204) sequences. However, this oligonucleotide almost certainly also hybridizes to mRNA encoding the EST isoform represented by rEST-6 (Falany *et al.*, 1995) (GenBank accession number S76490) and STe2 (Rikke and Roy, 1996) (GenBank accession number U50205), because these EST mRNA sequences and the oligonucleotide probe differ by only one nucleotide. It would be difficult to design any hybridization oligonucleotide capable of discriminating among the closely related EST isoforms, because sequence alignments reveal no ~20-base runs displaying more than three nucleotide differences.

The nucleotide sequence of the SULT-Dopa/tyrosine isoform, which sulfates dopamine and tyrosine residues (Sakakibara *et al.*, 1995; Araki *et al.*, 1997), is almost identical to that of the recently reported SULT1B1 (or ST1B1) (GenBank accession number D89375) (Fujita *et al.*, 1997), which catalyzes the sulfation of 3,3′,5′-triiodothyronine hormone (Yamazoe *et al.*, 1994). The coding regions of these two cDNAs differ by only one nucleotide, resulting in an amino acid substitution.

Analysis of the deduced amino acid sequences of SULT2 gene subfamily members suggests that rat and human SULT2 enzymes share ~62% amino acid sequence identity with each other and ~33–39% sequence similarity with the SULT1 gene subfamily members (Yamazoe *et al.*, 1994). In 1989, Ogura *et al.* screened a rat liver cDNA library with a polyclonal antibody prepared against female-predominant SULT2 (STa) and they cloned ST-20 (SULT20) cDNA (Ogura *et al.*, 1989) and ST-40 (SULT40) cDNA, which shared ~94.4% nucleotide sequence identity with SULT20 cDNA (Ogura *et al.*, 1990a). Subsequently, the SULT21, SULT41, and SULT60 cDNA sequences were identified by rat liver cDNA library screening (Watabe *et al.*, 1994). Comparative analysis of the deduced amino acid sequences for the protein subunits encoded by the rat hepatic SULT2 cDNAs suggests that the SULT20, -21, -40, -41, and -60 deduced protein sequences are structurally very similar, with sequence identities ranging from ~86.3 to ~99.6% (Watabe *et al.*, 1994).

In the liver, the functional significance of microheterogeneity within SULT gene subfamilies is uncertain. For example, SULT20 cDNA differs from SULT21 cDNA by eight nucleotides or six amino acid residues (Watabe *et al.*, 1994), whereas SULT40 and SULT41 differ from each other by only three nucleotide residues or one amino acid (Watabe *et al.*, 1994). To add to the complexity, SULT21 consists of two heterogeneous cDNAs, *i.e.* ST-21a (SULT21a) and ST-21b (SULT21b), which differ from each other only in the length of their 3′-untranslated regions (Watabe *et al.*, 1994).

As a consequence of opposing effects on individual SULT1 isoforms, the net effects of PB on SULT1 gene subfamily expression, as detected with a SULT1 cDNA probe, were not striking (fig. 1). Similarly, the net results of SULT2 Northern blot and Western blot analyses using a SULT2 cDNA probe and polyclonal antisera, respectively, suggested an overall suppressive effect of PB on SULT2 gene expression. In a previous study of the effects of tamoxifen and PB treatment on drug-metabolizing enzyme gene expression, Northern blot analyses of male rat liver total RNA hybridized with selected SULT cDNA probes suggested that PB increased rSTe1 (SULT1E2) mRNA expression and suppressed rSTa (SULT2) mRNA levels (Hell-

riegel *et al.,* 1996). The present study establishes, using isoform-specific oligonucleotide probes, that PB treatment produces markedly divergent effects on the mRNA expression of individual SULT isoforms, an effect that may be masked by the use of class-specific cDNA probes in Northern blot analyses.

Despite the strong ~94.4% nucleotide sequence identity shared by the cDNAs corresponding to SULT20 (SULT20/21 isoform) and SULT40 (SULT40/41 isoform) (Ogura *et al.,* 1989, 1990a), PB suppressed SULT20/21 mRNA levels and substantially induced SULT40/41 mRNA expression in rat liver. Like SULT40/41, hepatic SULT60 mRNA levels were increased in response to PB, suggesting that the subtle PB-mediated changes in SULT2 mRNA expression (detected in Northern blots using class-specific SULT2 cDNA probes) conceal more striking and even opposing effects on individual SULT2 isoforms. Noncoordinate pretranslational regulation of the hepatic expression of SULT20/21 and SULT40/41 isoforms in rat liver was previously reported (Ueda *et al.,* 1997). In growth hormone-deficient rats, SULT20/21 (called ST2A1) mRNA levels became undetectable in the livers of both male and female rats, whereas SULT40/41 (called ST2A2) mRNA expression was clearly detectable (Ueda *et al.,* 1997). Delivery of growth hormone by continuous infusion to hypophysectomized rats (female-specific secretory pattern) produced increases in hepatic SULT20/21 mRNA levels but had no significant effects on SULT40/41 expression in male or female rats (Ueda *et al.,* 1997). In contrast, treatment of growth hormone-deficient rats with intermittent injections of growth hormone (male-specific secretory pattern) resulted in a slight increase in hepatic SULT20/21 mRNA expression in mature male rats, no significant effects on SULT20/21 mRNA levels in mature female rats, and marked suppression of hepatic SULT40/41 mRNA expression in both male and female rats (Ueda *et al.,* 1997). These data suggest that gender-dependent growth hormone secretory patterns differentially regulate the hepatic mRNA expression of two highly related SULT2 isoforms (SULT20/21 and SULT40/41).

PB may alter SULT mRNA expression by suppressing or inducing the rates of SULT gene transcription or by modifying SULT mRNA stability. To add to this complexity, it is possible that PB regulates the molecular expression of different SULT isoforms by more than one mechanism, as has been suggested by previous investigations of PB-inducible P450 expression (Burger *et al.,* 1990; Kocarek *et al.,* 1990). Unlike the well-described aryl hydrocarbon receptor-mediated mechanism for the 2,3,7,8-tetrachlorodibenzo-*p*-dioxin-mediated induction of CYP1A1 gene transcription (Whitlock, 1993), enantioselectivity studies have failed to identify a stereospecific "PB receptor" (James *et al.,* 1981; Nims *et al.,* 1994). However, a 163-base pair fragment of the CYP2B2 5′-flanking region that was found to confer PB responsiveness to a reporter construct transiently transfected into primary cultured rat hepatocytes was recently identified (Trottier *et al.,* 1995). The molecular mechanisms and cellular signaling pathways that mediate the regulation of SULT mRNA expression by PB remain to be elucidated. Future studies addressing this problem will necessarily focus on the ability of PB, structural congeners of PB, and structurally dissimilar, PB-like, CYP2B1/2 inducers to modulate the rates of transcription of individual SULT isoforms in rat liver.

References

Araki Y, Sakakibara Y, Boggaram V, Katafuchi J, Suiko M, Nakajima H and Liu M-C (1997) Tissue-specific and developmental stage-dependent expression of a novel rat dopa/tyrosine sulfotransferase. *Int J Biochem Cell Biol* **29:**801–806.

Burger HJ, Schuetz EG, Schuetz JD and Guzelian PS (1990) Divergent effects of cycloheximide on the induction of class II and class III cytochrome P450 mRNAs in cultures of adult rat hepatocytes. *Arch Biochem Biophys* **281:**204–211.

Collins ML and Hunsaker WR (1985) Improved hybridization assays employing tailed oligonucleotide probes: a direct comparison with 5′-end-labeled oligonucleotide probes and nick-translated plasmid probes. *Anal Biochem* **151:**211–224.

Demyan WF, Song CS, Kim DS, Her S, Gallwitz W, Rao TR, Slomczynska M, Chatterjee B and Roy AK (1992) Estrogen sulfotransferase of the rat liver: complementary DNA cloning and age- and sex-specific regulation of messenger RNA. *Mol Endocrinol* **6:**589–597.

Duffel MW, Binder TP, Hosie L, Baden HA, Sanders JA, Knapp SA and Baron J (1991) Purification, immunochemical characterization, and immunohistochemical localization of rat hepatic aryl sulfotransferase IV. *Mol Pharmacol* **40:**36–44.

Falany JL, Krasnykh V, Mikheeva G and Falany CN (1995) Isolation and expression of an isoform of rat estrogen sulfotransferase. *J Steroid Biochem Mol Biol* **52:**35–44.

Fujita K-i, Nagata K, Ozawa S, Sasano H and Yamazoe Y (1997) Molecular cloning and characterization of rat ST1B1 and human ST1B2 cDNAs, encoding thyroid hormone sulfotransferases. *J Biochem (Tokyo)* **122:**1052–1061.

Hellriegel ET, Matwyshyn GA, Fei P, Dragnev KH, Nims RW, Lubet RA and Kong AN (1996) Regulation of gene expression of various phase I and phase II drug-metabolizing enzymes by tamoxifen in rat liver. *Biochem Pharmacol* **52:**1561–1568.

Hirshey SJ, Dooley TP, Reardon IM, Heinrikson RL and Falany CN (1992) Sequence analysis, *in vitro* translation, and expression of the cDNA for rat liver minoxidil sulfotransferase. *Mol Pharmacol* **42:**257–264.

Hirshey SJ and Falany CN (1990) Purification and characterization of rat liver minoxidil sulphotransferase. *Biochem J* **270:**721–728.

Jakoby WB, Sekura RD, Lyon ES, Marcus CJ and Wang J-L (1980) Sulfotransferases, in *Enzymatic Basis of Detoxication* (Jakoby WB ed) pp 199–228, Academic Press, New York.

James R, Kupfer A, Villeneuve JP and Branch RA (1981) Induction of drug-metabolizing enzymes by the enantiomers of normephenytoin in the rat. *Drug Metab Dispos* **9:**297–302.

Kocarek TA, Schuetz EG and Guzelian PS (1990) Differentiated induction of cytochrome P450b/e and P450p mRNAs by dose of phenobarbital in primary cultures of adult rat hepatocytes. *Mol Pharmacol* **38:**440–444.

Laemmli UK (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature (Lond)* **227:**680–685.

Liu L and Klaassen CD (1996a) Regulation of hepatic sulfotransferases by steroidal chemicals in rats. *Drug Metab Dispos* **24:**854–858.

Liu L and Klaassen CD (1996b) Ontogeny and hormonal basis of male-dominant rat hepatic sulfotransferases. *Mol Pharmacol* **50:**565–572.

Liu L and Klaassen CD (1996c) Ontogeny and hormonal basis of female-dominant rat hepatic sulfotransferases. *J Pharmacol Exp Ther* **279:**386–391.

Liu L, LeCluyse EL, Liu J and Klaassen CD (1996) Sulfotransferase gene expression in primary cultures of rat hepatocytes. *Biochem Pharmacol* **52:**1621–1630.

Lubet RA, Dragnev KH, Chauhan DP, Nims RW, Diwan BA, Ward JM, Jones CR, Rice JM and Miller MS (1992) A pleiotropic response to phenobarbital-type enzyme inducers in the F344/NCr rat. *Biochem Pharmacol* **43:**1067–1078.

Meerman JHN, Beland FA and Mulder GJ (1981) Role of sulfation in the formation of DNA adducts from *N*-hydroxy-2-acetylaminofluorene in rat liver *in vivo:* inhibition of *N*-acetylated aminofluorene adduct formation by pentochlorophenol. *Carcinogenesis (Lond)* **2:**413–416.

Nagata K, Ozawa S, Miyata M, Shimada M, Gong D-W, Yamazoe Y and Kato R (1993) Isolation and expression of a cDNA encoding a male-specific rat sulfotransferase that catalyzes activation of *N*-hydroxy-2-acetylaminofluorene. *J Biol Chem* **268:**24720–24725.

Nims RW, Sidhu JS, Thomas PE, Mellini DW, Nelson VC, Omiecinski CJ and Lubet RA (1994) Absence of enantioselectivity in the pharmacodynamics of P450 2B induction in the male rat liver or in cultured rat hepatocytes. *J Biochem Toxicol* **9:**279–288.

Ogura K, Kajita J, Narihata H, Watabe T, Ozawa S, Nagata K, Yamazoe Y and Kato R (1989) Cloning and sequence analysis of a rat liver cDNA encoding hydroxysteroid sulfotransferase. *Biochem Biophys Res Commun* **165:**168–174.

Ogura K, Kajita J, Narihata H, Watabe T, Ozawa S, Nagata K, Yamazoe Y and Kato R (1990a) cDNA cloning of the hydroxysteroid sulfotransferase STa sharing a strong homology in amino acid sequence with the senescence marker protein SMP-2 in rat livers. *Biochem Biophys Res Commun* **166:**1494–1500.

Ogura K, Sohtome T, Sugiyama A, Okuda H, Hiratsuka A and Watabe T (1990b) Rat liver cytosolic hydroxysteroid sulfotransferase (sulfotransferase a) catalyzing the formation of reactive sulfate esters from carcinogenic polycyclic hydroxymethylarenes. *Mol Pharmacol* **37:**848–854.

Okuda H, Nojima N, Watanabe N and Watabe T (1989) Sulfotransferase-mediated activation of the carcinogen 5-hydroxymethyl-chrysene: species and sex differences in tissue distribution of the enzyme activity and a possible participation of hydroxysteroid sulfotransferases. *Biochem Pharmacol* **38:**3003–3009.

Ozawa S, Nagata K, Gong D, Yamazoe Y and Kato R (1990) Nucleotide sequence of a full-length cDNA (PST-1) for aryl sulfotransferase from rat liver. *Nucleic Acids Res* **18:**4001.

Puissant C and Houdebine L-M (1990) An improvement of the single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Biotechniques* **8:**148–149.

Rikke BA and Roy AK (1996) Structural relationships among members of the mammalian sulfotransferase gene family. *Biochim Biophys Acta* **1307:**331–338.

Runge-Morris MA (1994) Sulfotransferase gene expression in rat hepatic and extrahepatic tissues. *Chem Biol Interact* **92:**67–76.

Runge-Morris M, Rose K and Kocarek TA (1996) Regulation of rat hepatic sulfotransferase gene expression by glucocorticoid hormones. *Drug Metab Dispos* **24:**1095–1101.

Runge-Morris M and Vento C (1994) Effect of phenobarbital and dexamethasone treatment on rat liver hydroxysteroid sulfotransferase gene expression. *FASEB J* **8:**A1251.

Runge-Morris M and Vento C (1995) Effects of streptozotocin-induced diabetes on rat liver sulfotransferase gene expression. *Drug Metab Dispos* **23:**455–459.

Runge-Morris M and Wilusz J (1991) Age- and gender-related gene expression of hydroxysteroid sulfotransferase-a in rat liver. *Biochem Biophys Res Commun* **175:**1051–1056.

Runge-Morris M and Wilusz J (1994) Suppression of hydroxysteroid sulfotransferase-a gene expression by 3-methylcholanthrene. *Toxicol Appl Pharmacol* **125:**133–141.

Sakakibara Y, Takami Y, Zwieb C, Nakayama T, Suiko M, Nakajima H and Liu M-C (1995) Purification, characterization, and molecular cloning of a novel rat liver dopa/tyrosine sulfotransferase. *J Biol Chem* **270:**30470–30478.

Singer SS and Sylvester S (1976) Enzymatic sulfation of steroids. II. The control of the hepatic cortisol sulfotransferase activity and of the individual hepatic steroid sulfotransferases of rats by gonads and gonadal hormones. *Endocrinology* **99:**1346–1352.

Smith PK, Krohn RI, Hermanson GT, Mallia AK, Gartner FH, Provenzano MD, Fujimoto EK, Goeke NM, Olson BJ and Klenk DC (1985) Measurement of protein using bicinchoninic acid. *Anal Biochem* **150:**76–85.

Song CS, Kim JM, Roy AK and Chatterjee B (1990) Structure and regulation of the senescence marker protein 2 gene promoter. *Biochemistry* **29:**542–551.

Trottier E, Belzil A, Stoltz C and Anderson A (1995) Localization of a phenobarbital-responsive element (PBRE) in the 5′-flanking region of the rat CYP2B2 gene. *Gene* **158:**263–268.

Ueda R, Shimada M, Hashimoto H, Ishikawa H and Yamazoe Y (1997) Distinct regulation of two hydroxysteroid sulfotransferases, ST2A1 and ST2A2, by growth hormone: a unique type of growth hormone regulation in rats. *J Pharmacol Exp Ther* **282:**1117–1121.

Watabe T, Ogura K, Satsukawa M, Okuda H and Hiratsuka A (1994) Molecular cloning and functions of rat liver hydroxysteroid sulfotransferases catalysing covalent binding of carcinogenic polycyclic arylmethanols to DNA. *Chem Biol Interact* **92:**87–105.

Whitlock JP Jr (1993) Mechanistic aspects of dioxin action. *Chem Res Toxicol* **6:**754–763.

Yamazoe Y, Nagata K, Ozawa S and Kato R (1994) Structural similarity and diversity of sulfotransferases. *Chem Biol Interact* **92:**107–117.

Yerokun T, Etheredge JL, Norton TR, Carter HA, Chung KH, Birckbichler PJ and Ringer DP (1992) Characterization of a complementary DNA for rat liver aryl sulfotransferase IV and use in evaluating the hepatic gene transcript levels of rats at various stages of 2-acetylaminofluorene-induced hepatocarcinogenesis. *Cancer Res* **52:**4779–4786.