J. Biochem. **137**, 401–406 (2005)
DOI: 10.1093/jb/mvi047

Oral Contraceptives as Substrates and Inhibitors for Human Cytosolic SULTs

Shin Yasuda, Masahito Suiko and Ming-Cheh Liu*

Biomedical Research Center, The University of Texas Health Center, 11937 U.S. Highway 271, Tyler, TX 75708, USA

Received November 14, 2004; accepted January 11, 2005

Cytosolic sulfotransferases (SULTs) in mammals are involved in the biotransformation and homeostasis of various endogenous and xenobiotic compounds. The current study aimed to examine the sulfation of contraceptive compounds by various human cytosolic SULTs and to investigate the inhibitory effects and mode of action of these compounds on the sulfation of 17β-estradiol, a major endogenous estrogen. A systematic study using all eleven known human cytosolic SULTs revealed the differential substrate specificity of these enzymes for the eight representative contraceptive compounds and two endogenous estrogens (estrone and 17β-estradiol) tested as substrates. Activity data showed that SULT1A1 displayed the strongest activity toward 17α-ethynylestradiol. Kinetic studies revealed that the V_{max} value of the sulfation of 17α-ethynylestradiol by SULT1A1 was 1.64 times that of the sulfation of 17β-estradiol, while the K_m values were almost equal for the two compounds. The inhibitory effects of three contraceptive compounds on the sulfation of 17β-estradiol by SULT1A1 were examined. IC_{50} values determined were 0.193, 1.84, and 2.98 mM, respectively, for 19-norethindrone acetate, ethynodiol diacetate and mifepristone. Kinetic analyses indicated that the mechanism underlying the inhibition by these contraceptives is of a mixed noncompetitive type. Metabolic labeling experiments confirmed the sulfation of contraceptive compounds and the release of their sulfated derivatives by HepG2 human hepatoma cells. Collectively, the results obtained suggest a role of sulfation in the metabolism of contraceptive compounds *in vivo*. Moreover, in view of their inhibitory effects on the sulfation of 17β-estradiol, these compounds may potentially act to disrupt the homeostasis of endogenous estrogens.

Key words: contraceptive, 17α-ethynylestradiol, 17β-estradiol, phenol sulfotransferase, sulfation.

Abbreviations: SULT, sulfotransferase; PAPS, 3′-phosphoadenosine 5′-phosphosulfate; SDS-PAGE, sodium dodecyl sulfate–polyacrylamide gel electrophoresis.

Sulfate conjugation is a major pathway in mammals for the biotransformation and excretion of drugs/xenobiotics as well as the homeostasis of endogenous compounds such as steroid and thyroid hormones, catecholamines, cholesterol, and bile acids (*1–3*). The responsible enzymes, called the "cytosolic sulfotransferases (SULTs)," catalyze the transfer of a sulfonate group from the active sulfate, 3′-phosphoadenosine 5′-phosphosulfate (PAPS), to an acceptor substrate compound containing a hydroxyl or an amino group (*4*). Sulfate conjugation by these enzymes may result in the inactivation (or, in some cases, activation) of the substrate compounds and/or increase their water-solubility, thereby facilitating their removal from the body (*1–3*).

We have recently begun investigating the involvement of sulfation in the metabolism and adverse functioning of environmental xenoestrogens (*5*, *6*). These compounds, including natural sterols, pesticides, pollutants, and synthetic compounds, have in recent years been increasingly recognized as a potential hazardous factor for human health (*7*). They are not only suspected of causing developmental abnormalities in wildlife but are also being cited as a serious hazard for human health (*7–10*). A worldwide debate on a decline in sperm quality in men (*11*, *12*) and increased incidences of human breast cancer (*13–15*) implicate xenoestrogens as the putative cause for these epidemiological observations. We recently demonstrated that human cytosolic SULTs, in particular the simple phenol (P)-form SULT (SULT1A1), can catalyze the sulfation of some representative environmental xenoestrogens (*5*, *6*). To what extent the sulfation is involved in the inactivation and/or excretion of these compounds, however, remains to be clarified. Another group of xenoestrogen compounds gaining increased attention are the oral contraceptives (*16–18*). These medical xenoestrogens, while providing desired birth control effects, have been linked to an increased risk of breast cancer (*16–18*). The various compounds used in oral contraceptives are derivatives of 17β-estradiol or progesterone, and many of them contain hydroxyl groups (*19*). It is therefore tempting to question whether sulfate conjugation of these contraceptive compounds may take place

*To whom correspondence should be addressed. Tel: +1-903-877-2862, Fax: +1-903-877-2863, E-mail: ming.liu@uthct.edu

and whether sulfation occurs *in vivo* to counteract the adverse effects of (excess) oral contraceptives. Moreover, in view of their structural similarity to 17β-estradiol, are these contraceptive compounds capable of inhibiting the sulfation of 17β-estradiol, thereby disrupting the homeostasis of this important endogenous estrogen *in vivo*?

We report here a systematic investigation of the differential activities of the eleven known human cytosolic SULTs toward estrogenic compounds commonly present in oral contraceptives. The kinetics of the sulfation of 17β-estradiol and 17α-ethynylestradiol by SULT1A1, the major cytosolic SULT demonstrated to be involved in the sulfation of contraceptive compounds, were examined. The inhibitory effects of 19-norethindrone acetate, ethynodiol diacetate and mifepristone on the 17β-estradiol–sulfating activity of SULT1A1 and the underlying mechanism of inhibition were studied. Moreover, using HepG2 human hepatoma cells as a model, the metabolism of contraceptive compounds through sulfation and the release of their sulfated derivatives were investigated.

MATERIALS AND METHODS

Materials—Estrone, 17β-estradiol, 17α-ethynylestradiol, 19-norethindrone, 19-norethindrone acetate, ethynodiol diacetate, levonorgestrel, medroxyprogesterone, mifepristone, aprotinin, thrombin, adenosine 5′-triphosphate (ATP), PAPS, sodium dodecyl sulfate (SDS), Trizma base, isopropyl-β-D-thiogalactopyranoside (IPTG), inorganic pyrophosphatase, dithiothreitol (DTT), and dimethyl sulfoxide (DMSO) were obtained from Sigma Chemical Co. (St. Louis, MO). Mestranol was obtained from MP Biomedicals, Inc. (Aurora, OH). Carrier-free sodium [^{35}S]sulfate was from ICN Biomedicals Inc. (Irvine, CA). Cellulose thin-layer chromatography (TLC) plates were from EMD Chem. Inc. (Gibbstown, NJ). HepG2 human hepatoma cell line (ATCC HB 8065) was from American Type Culture Collection. All other chemicals were of the highest grade commercially available.

Preparation of Purified Human Cytosolic SULTs—Recombinant human P-form (SULT1A1 and SULT1A2) and M-form (SULT1A3) phenol SULTs, thyroid hormone SULT (SULT1B2), two SULT1Cs (designated #1 and #2), estrogen SULT (SULT1E1), dehydroepiandrosterone (DHEA) SULT (SULT2A1), two SULT2B1s (designated a and b), and a neuronal SULT (SULT4A1), expressed using pGEX-2TK or pET23c prokaryotic expression system, were prepared as previously described (*5*, *6*, *20–22*).

Enzymatic Assay—The sulfating activity of the recombinant human cytosolic SULTs was assayed using PAP[^{35}S] as the sulfonate group donor. The standard assay mixture, in a final volume of 25 μl, contained 50 mM Mops buffer at pH 7.5, 1 mM DTT, and 14 μM PAP[^{35}S]. The substrate, dissolved in DMSO at 10 times the final concentration in the assay mixture, was added after Mops buffer and PAP[^{35}S]. Control with DMSO alone was also prepared. The reaction was started by the addition of the enzyme (1.25 μg), allowed to proceed for 3 min at 37°C, and stopped by placing the assay mixture-containing thin-wall tube on a heating block, pre-heated to 100°C, for 2 min. The precipitates were cleared by centrifugation for 1 min, and the supernatant was subjected to the analysis of [^{35}S]sulfated product using the previ-

Table 1. **Specific activities of the human cytosolic SULTs with endogenous estrogens and contraceptive compounds as substrates.***

	Specific activity (nmol/min/mg)									
Substrate	SULT1A1	SULT1A2	SULT1A3	SULT1B2	SULT1C#1	SULT1C#2	SULT1E1	SULT2A1	SULT2B1a	SULT2B1b	SULT4A1
Estrone	0.32 ± 0.02	0.13 ± 0.01	0.16 ± 0.01	ND	ND	0.87 ± 0.02	5.89 ± 0.27	0.18 ± 0.01	ND	ND	ND
17β-Estradiol	5.20 ± 0.13	1.97 ± 0.07	3.94 ± 0.07	ND	ND	5.56 ± 0.21	6.85 ± 0.14	1.47 ± 0.03	ND	ND	ND
17α-EE	12.35 ± 0.92	0.56 ± 0.04	0.25 ± 0.01	ND	ND	0.97 ± 0.06	4.65 ± 0.06	1.11 ± 0.05	ND	ND	ND
19-Nored	ND	ND	ND	ND	ND	ND	0.17 ± 0.01	0.62 ± 0.02	ND	ND	ND
19-Nored acetate	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Ethynodiol diacetate	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Levonorgestrel	ND	ND	ND	ND	ND	ND	ND	0.08 ± 0.01	ND	ND	ND
Mestranol	ND	ND	ND	ND	ND	ND	ND	0.12 ± 0.01	ND	ND	ND
Medroxyprogesterone	0.31 ± 0.01	ND	ND	ND	ND	0.19 ± 0.00	ND	ND	ND	ND	ND
Mifepristone	0.35 ± 0.02	ND	ND	ND	ND	0.17 ± 0.01	ND	ND	ND	ND	ND

*The assay mixture contained 1 mM DTT, 14 μM PAPS, 100 μM of substrate and 50 mM Mops buffer, pH 7.5. The assays were carried out at 37°C for 3 min. Data are the mean ± SD from three determinations. ND refers to activity not detected. SULT, sulfotransferase; 17α-EE, 17α-ethynylestradiol; 19-Nored, 19-norethindrone.

Table 2. **Kinetic constants of the human SULT1A1 with 17β-estradiol and 17α-ethynylestradiol as substrates.***

Substrate	V_{max} (nmol/min/mg)	K_m (μM)	V_{max}/K_m
17β-Estradiol	7.29	3.54	2.06
17α-Ethynylestradiol	11.98	3.43	3.49

*The assay mixture contained 1 mM DTT, 14 μM PAPS, varying concentrations of substrate and 50 mM Mops buffer, pH 7.5. The assays were carried out at 37°C for 3 min. Data are the mean values from three determinations.

ously established TLC procedure (*23*), with *n*-butanol/isopropanol/88% formic acid/water (3:1:1:1; by volume) as the solvent system. Each experiment was performed in triplicate, together with a control without enzyme. The results obtained were calculated and expressed in nanomoles of sulfated product formed/min/mg purified enzyme.

Metabolic Labeling of HepG2 Human Hepatoma Cells—HepG2 cells were routinely maintained, under a 5% CO_2 atmosphere at 37°C, in minimum essential medium (MEM) supplemented with 10% fetal bovine serum, penicillin G (30 μg/ml), and streptomycin sulfate (50 μg/ml). Confluent HepG2 cells grown in individual wells of a 24-well culture plate, preincubated in sulfate-free (prepared by omitting streptomycin sulfate and replacing magnesium sulfate with magnesium chloride) MEM for 4 h, were labeled with 0.2-ml aliquots of the same medium containing [^{35}S]sulfate (0.3 mCi/ml) and different concentrations (ranging from 5 to 100 μM) of representative compounds present in oral contraceptives. At the end of an 18-h labeling, the media were collected, spin-filtered, and subjected to TLC analysis for [^{35}S]sulfated compounds based on the above-mentioned procedure (*23*).

Miscellaneous Methods—PAP[^{35}S] was synthesized from ATP and carrier-free [^{35}S]sulfate using the bifunctional human ATP sulfurylase/adenosine 5′-phosphosulfate kinase, and its purity was determined as previously described (*24*). The PAP[^{35}S] synthesized was adjusted to the required concentration and specific activity of 15 Ci/mmol at 1.4 mM by the addition of cold PAPS. SDS–polyacrylamide gel electrophoresis was performed on 12% polyacrylamide gels using the method of Laemmli (*25*). Protein determination was based on the method of Bradford with bovine serum albumin as the standard (*26*).

RESULTS AND DISCUSSION

The current study aimed to examine the sulfation of contraceptive compounds by various human cytosolic SULTs and to investigate the inhibitory effects and mode of action of these compounds on the sulfation of 17β-estradiol, a major endogenous estrogen.

Differential Sulfating Activities of the Human Cytosolic SULTs toward Contraceptive Compounds—We have previously cloned, expressed, and purified all eleven known human cytosolic SULTs: SULT1A1, SULT1A2, SULT1A3, SULT1B2, SULT1C#1, SULT1C#2, SULT1E1, SULT2A1, SULT2B1a SULT2B1b, and SULT4A1 (*5*, *6*, *20–22*). These purified recombinant human cytosolic SULTs were examined for sulfating activities toward representative compounds present in oral contraceptives, namely 17α-ethynylestradiol, 19-norethindrone, 19-norethindrone acetate, ethynodiol diacetate, levonorgestrel, mestranol, medroxyprogesterone and mifepristone, as well as the two major endogenous estrogens, estrone and 17β-estradiol. The results compiled in Table 1 showed that five (SULT1B2, SULT1C#1, SULT2B1a, SULT2B1b and SULT4A1) of the eleven SULTs displayed no detectable activities. The other six human cytosolic SULTs (SULT1A1, SULT1A2, SULT1A3, SULT1C#2, SULT1E1 and SULT2A1) exhibited differential sulfating activities toward the different compounds tested. Since SULT1E1 is the major human cytosolic SULT responsible for the sulfate conjugation of endogenous estrogens *in vivo* (*27*, *28*), it is not surprising that the enzyme displayed the strongest activities toward the two endogenous estrogens, 17β-estradiol and estrone. With 17α-ethynylestradiol (a major component of many combined oral contraceptives) as substrate, however, SULT1A1 displayed the strongest sulfating activity. Of the other seven contraceptive compounds tested, five (19-norethindrone, mestranol, levonorgestrel, medroxyprogesterone, and mifepristone) were sulfated at low levels by SULT1A1, SULT1C#2,

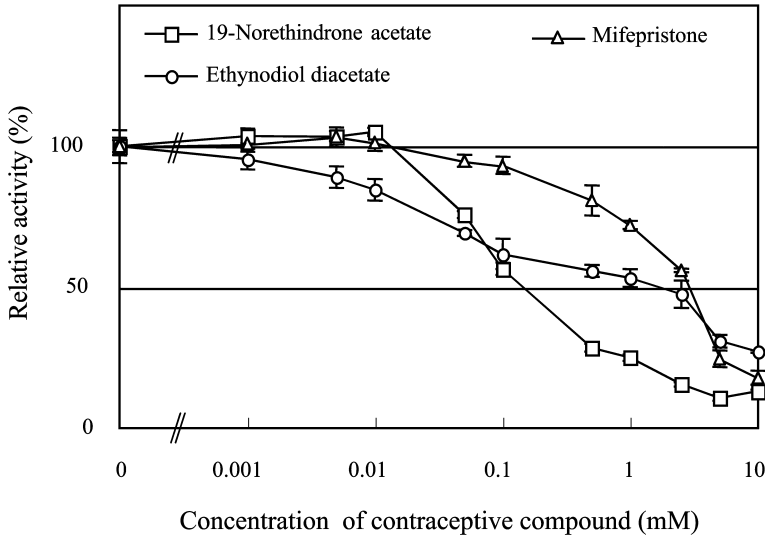

Fig. 1. **Inhibitory effects of 19-norethindrone acetate, ethynodiol diacetate and mifepristone on the sulfation of 17β-estradiol by SULT1A1.** Enzymatic assays with 5 μM 17 β-estradiol as substrate were performed in the presence of varying concentrations of 19-norethindrone acetate, ethynodiol diacetate, or mifepristone. Data from three experiments were calculated based on the activity determined in the absence of these compounds as 100%.

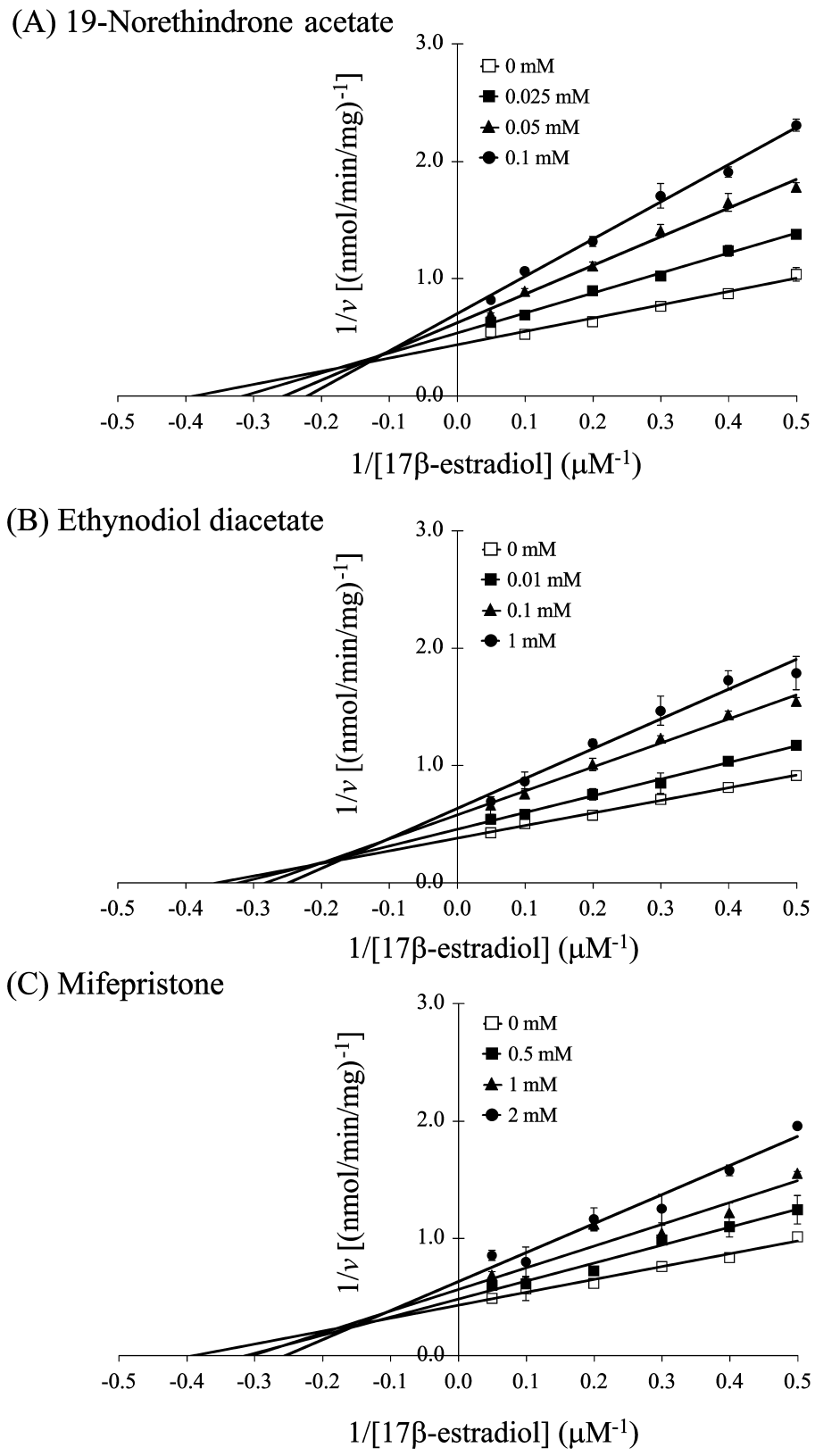

Fig. 2. **Lineweaver-Burk double-reciprocal plots of SULT1A1 with 17β-estradiol as substrate in the presence of different concentrations of 19-norethindrone acetate (A), ethynodiol diacetate (B), and mifepristone (C).** Concentrations of these contraceptive compounds tested were 0, 0.025, 0.05 and 0.1 mM in (A); 0, 0.01, 0.1 and 1 mM in (B); and 0, 0.5, 1 and 2 mM in (C). Concentrations of 17β-estradiol were in μM and velocities were expressed as nanomoles of sulfated product formed/min/mg protein. Data shown represent mean ± SD derived from three independent determinations.

SULT1E1, and SULT2A1, and the other two (19-norethindrone acetate and ethynodiol diacetate) were not sulfated. The kinetic parameters of SULT1A1 in catalyzing the sulfation of 17α-ethynylestradiol and 17β-estradiol were further investigated. The enzymatic assays were performed at pH 7.5 with varying concentrations of these two compounds as substrates. Results compiled in Table 2 revealed that, while the K_m values for the two compounds were nearly identical (3.54 μM vs. 3.43 μM), the V_{max} with 17α-ethynylestradiol as substrate (11.98 nmol/

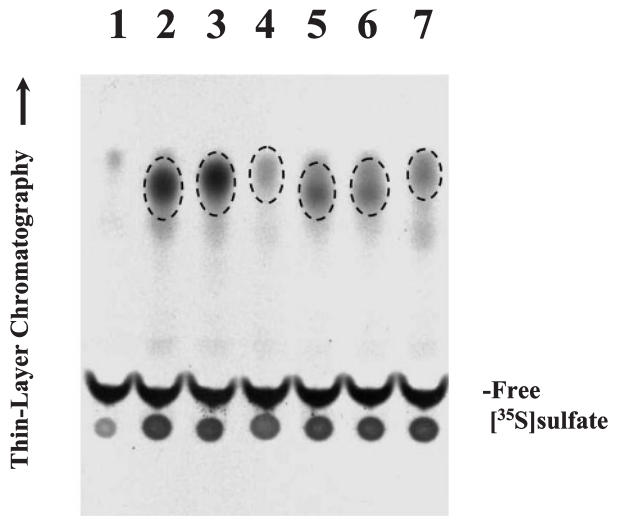

Fig. 3. **Analysis of [^{35}S]sulfated products generated and released by HepG2 human hepatoma cells labeled with [^{35}S]sulfate in the presence of contraceptive compounds.** The figure shows the autoradiograph taken from the TLC plate at the end of the analysis. The compounds, tested at 5 μM concentrations in the labeling media, were 17β-estradiol (lane 2), 17α-ethynylestradiol (lane 3), levonorgestrel (lane 4), 19-norethindrone (lane 5), 19-norethindrone acetate (lane 6), and mestranol (lane 7). Lane 1 shows the control with no additional compound in the labeling medium.

min/mg) was considerably higher than that with 17β-estradiol as substrate (7.29 nmol/min/mg). Catalytic efficiency of SULT1A1, as reflected by V_{max}/K_m, therefore appeared to be higher with 17α-ethynylestradiol than with 17β-estradiol as substrate. Because SULT1A1 is expressed in the liver and gastrointestinal tract as well as blood platelets (*29–31*), these results may implicate SULT1A1 in the metabolism, and possibly inactivation, of 17α-ethynylestradiol and some other compounds used in oral contraceptives. It should also be noted that, while sulfation of these compounds may lead to their inactivation and disposal, it may at the same time suppress the sulfation of other compounds. In the case of 17β-estradiol, the presence of, for example, 17α-ethynylestradiol may interfere with its sulfation, leading to the prolonged presencee (in an unconjugated state) and estrogenic action of 17β-estradiol.

Inhibitory Effects of Contraceptive Compounds on the Sulfation of 17β-Estradiol by the Human Cytosolic SULTs—Previous studies have indicated that some xenobiotics may act as inhibitors of SULT enzymes (*32*). In a preliminary experiment, we noticed that three compounds (19-norethindrone acetate, ethynodiol diacetate and mifepristone) that were poor substrates for SULT enzymes (see Table 1), could exert inhibitory effects on the sulfation of 17β-estradiol by SULT1A1 (data not shown). To investigate further this interesting phenomenon, enzymatic assays were performed using 5 μM 17β-estradiol as the substrate in the presence of varying concentrations (ranging from 0 to 10 mM) of 19-norethindrone acetate, ethynodiol diacetate, or mifepristone. Activity data obtained revealed a concentration-dependent inhibition of the 17β-estradiol–sulfating activity of the SULT1A1 by all three compounds. Based on the results compiled in Fig. 1, the IC_{50} values determined for 19-norethindrone acetate, ethynodiol diacetate, and mifepristone were 0.193, 1.84 and 2.98 mM, respectively. We then proceeded to investigate the mechanism underlying the inhibition of the 17β-estradiol–sulfating activity of SULT1A1 by these compounds. Kinetic experiments were performed in the presence of fixed concentrations of 19-norethindrone acetate (0, 0.025, 0.05 and 0.1 mM), ethynodiol diacetate (0, 0.01, 0.1 and 1 mM), or mifepristone (0, 0.5, 1 and 2 mM). Data obtained were used to generate Lineweaver-Burk double reciprocal plots. As shown in Fig. 2, the lines corresponding to the various concentrations of 19-norethindrone acetate (Fig. 2A), ethynodiol diacetate (Fig. 2B), or mifepristone (Fig. 2C), while intersecting the *X*- and *Y*-axis at different positions, all appeared to converge within a narrow region in the figure. These results suggested a mixed noncompetitive type of inhibition whereby the binding of 19-norethindrone acetate, ethynodiol diacetate, or mifepristone not only decrease the catalytic activity of SULT1A1, but also interfere with the binding of 17β-estradiol as the substrate. As discussed previously, the inhibitory effects of these contraceptive compounds may disrupt the homeostasis of 17β-estradiol, leading to its prolonged presence and estrogenic action. It has been proposed that elevated levels of endogenous estrogens such as 17β-estradiol may be involved in the carcinogenic processes within the breast tissue (*33*).

Metabolic Studies on the Sulfation of Contraceptive Compounds—HepG2 human hepatoma cells were used to investigate whether the sulfation of contraceptive compounds occurs in a metabolic setting. Confluent HepG2 cells grown in wells of a 24-well plate were labeled with sulfate-free medium containing [^{35}S]sulfate and different concentrations of 17β-estradiol, 17α-ethynylestradiol, levonorgestrel, 19-norethindrone acetate, ethynodiol diacetate, and mestranol. As shown in Fig. 3, TLC analysis of medium samples collected at the end of an 18-h labeling period confirmed the presence of [^{35}S]sulfated products in medium samples containing concentrations as low as 5 μM of the compounds tested. It is interesting to note that, while levonorgestrel, 19-norethindrone, 19-norethindrone acetate, and mestranol were not good substrates for SULT1A1 *in vitro* (*cf.* Table 1), they may be metabolized (*e.g.*, hydroxylated by the action of cytochrome P-450) in the cells to become (better) substrates for sulfation *in vivo*.

In conclusion, the data presented in this communication represent the first systematic study on the sulfation of contraceptive compounds by human cytosolic SULTs. From the physiology standpoint, the fact that some of the contraceptive compounds tested can be sulfated, either directly or following other metabolic steps (*e.g.*, hydroxylation), suggests a role of sulfation in the metabolism of these compounds *in vivo*. Moreover, the finding that some contraceptive compounds may act to inhibit the sulfation of 17β-estradiol indicates a potential effect of these compounds in disrupting the homeostasis of endogenous estrogens. More work is warranted in order to fully elucidate these important issues.

We thank Kei Ohkimoto and Saki Takahashi for technical advice. This work was supported in part by funds from the

University of Texas Health Center at Tyler and a UTHCT President's Council Research Membership Seed Grant.

REFERENCES

1. Mulder, G.J. and Jakoby, W.B. (1990) Sulfation in *Conjugation Reactions in Drug Metabolism* (Mulder, G.J. and Jakoby, W.B., eds.) pp. 107–161, Taylor and Francis, London
2. Falany, C. and Roth, J.A. (1993) Properties of human cytosolic sulfotransferases involved in drug metabolism in *Human Drug Metabolism*; From Molecular Biology to Man (Jeffery, E.H., ed.) pp. 101 –115, CRC Press, Boca Raton, FL
3. Weinshilboum, R. and Otterness, D. (1994) Sulfotransferase enzymes in *Conjugation-Deconjugation Reactions in Drug Metabolism and Toxicity* (Kaufmann, F.C., ed.) pp. 45–78, Springer-Verlag, Berlin
4. Lipmann, F. (1958) Biological sulfate activation and transfer. *Science* **128**, 575–580
5. Suiko, M., Sakakibara, Y., and Liu, M.-C. (2000) Sulfation of environmental estrogen-like chemicals by human cytosolic sulfotransferases. *Biochem. Biophys. Res. Commun.* **267**, 80–84
6. Pai, T.G., Sugahara, T., Suiko, M., Sakakibara, Y., Xu, F., and Liu M.-C. (2002) Differential xenoestrogen-sulfating activities of the human cytosolic sulfotransferases: molecular cloning, expression, and purification of human SULT2B1a and SULT2B1b sulfotransferases. *Biochim. Biophys. Acta* **1573**, 165–170
7. Roy, D., Palangat, M., Chen, C.-W., Thomas, R.D., Colerangle, J., Atkinson, A., and Yan, Z.-J. (1997) Biochemical and molecular changes at the cellular level in response to exposure to environmental estrogen-like chemicals. *J. Toxicol. Environ. Health* **50**, 1–29
8. Guillette, L.J., Jr., Gross, T.S., Masson, G.R., Matter, J.M., Percival, H.F., and Woodward, A.R. (1994) Developmental abnormalities of the gonad and abnormal sex hormone concentrations in juvenile alligators from contaminated and control lakes in Florida. *Environ. Health Perspect.* **102**, 680–688
9. Fry, D.M. (1995) Reproductive effects in birds exposed to pesticides and industrial chemicals. *Environ. Health Prospect.* **103**, 165–171
10. Tyler, C.R., Jobling, S., and Sumpter, J.P. (1998) Endocrine disruption in wildlife: a critical review of the evidence. *Crit. Rev. Toxicol.* **28**, 319–361
11. Carlsen, E., Giwercman, A., Keiding, N., and Skakkebaek, N.E. (1992) Evidence for decreasing quality of semen during past 50 years. *Br. Med. J.* **305**, 609–613
12. Auger, J., Kunstmann, J.M., Czyglik, F., and Jouannet, P. (1995) Decline in semen quality among fertile men in Paris during the past 20 years. *New Engl. J. Med.* **332**, 281–285
13. Hoyer, A.P., Grandjean, P., Jorgensen, T., Brock, J.W., and Hartvig, H.B. (1998) Organochlorine exposure and risk of breast cancer. *Lancet* **352**, 1816–1820
14. Hoyer, A.P., Grandjean, P., Jorgensen, T., Brock, J.W., and Hartvig, H.B. (2000) Organochlorine compounds and breast cancer—is there a connection between environmental pollution and breast cancer? *Ugeskr Laeger* **162**, 922–926
15. Clarke, R., Hilakivi-Clarke, L., and Trock, B. (2001) Breast cancer: dietary and environmental oestrogens. *Biologist (London)* **48**, 21–26
16. Rookus, M.A. and van Leeuwen, F.E. (1994) Oral contraceptives and risk of breast cancer in women aged 20–54 years. Netherlands Oral Contraceptives and Breast Cancer Study Group. *Lancet* **344**, 844–851
17. La Vecchia, C., Altieri, A., Franceschi, S., and Tavani, A. (2001) Oral contraceptives and cancer: an update. *Drug Saf.* **24**, 741–754
18. Marchbanks, P.A., McDonald, J.A., Wilson, H.G., Folger, S.G., Mandel, M.G., Daling, J.R., Bernstein, L., Malone, K.E., Ursin, G., Strom, B.L., Norman, S.A., Wingo, P.A., Burkman, R.T., Berlin, J.A., Simon, M.S., Spirtas, R., and Weiss, L.K. (2002) Oral contraceptives and the risk of breast cancer. *N. Engl. J. Med.* **346**, 2025–2032
19. Petitti, D.B. (2003) Clinical practice. Combination estrogen-progestin oral contraceptives. *N. Engl. J. Med.* **349**, 1443–1450
20. Sakakibara, Y., Takami, Y., Nakayama, T., Suiko, M., and Liu M.-C. (1998) Localization and functional analysis of the substrate specificity/catalytic domains of human M-form and P-form phenol sulfotransferases. *J. Biol. Chem.* **273**, 6242–6247
21. Sakakibara, Y., Yanagisawa, K., Katafuchi, J., Ringer, D.P., Takami, Y., Nakayama, T., Suiko, M., and Liu, M.-C. (1998) Molecular cloning, expression, and characterization of novel human SULT1C sulfotransferases that catalyze the sulfonation of N-hydroxy-2-acetylaminofluorene. *J. Biol. Chem.* **273**, 33929–33935
22. Sakakibara, Y., Suiko, M., Pai, T.G., Nakayama, T., Takami, Y., Katafuchi, J., and Liu, M.-C. (2002) Highly conserved mouse and human brain sulfotransferases: molecular cloning, expression, and functional characterization. *Gene* **285**, 39–47
23. Liu, M.-C. and Lipmann, F. (1984) Decrease of tyrosine-O-sulfate-containing proteins found in rat fibroblasts infected with Rous sarcoma virus or Fujinami sarcoma virus. *Proc. Natl Acad. Sci. USA* **81**, 3695–3698
24. Yanagisawa, K., Sakakibara, Y., Suiko, M., Takami, Y., Nakayama, T., Nakajima, H., Takayanagi, K., Natori, Y., and Liu, M.-C. (1998) cDNA cloning, expression, and characterization of the human bifunctional ATP sulfurylase/adenosine 5′-phosphosulfate kinase enzyme. *Biosci. Biotechnol. Biochem.* **62**, 1037–1040
25. Laemmli, U.K. (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* **227**, 680–685
26. Bradford, M.M. (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* **72**, 248–254
27. Aksoy, I.A., Wood, T.C., and Weinshilboum, R. (1994) Human liver estrogen sulfotransferase: identification by cDNA cloning and expression. *Biochem. Biophys. Res. Commun.* **200**, 1621–1629
28. Falany, C.N., Krasnykh, V., and Falany, J.L. (1995) Bacterial expression and characterization of a cDNA for human liver estrogen sulfotransferase. *J. Steroid Biochem. Mol. Biol.* **52**, 529–539
29. Reiter, C. and Weinshilboum, R. (1982) Platelet phenol sulfotransferase activity: correlation with sulfate conjugation of acetaminophen. *Clin. Pharmacol. Ther.* **32**, 612–621
30. Heroux, J.A., Falany, C.N., and Roth, J.A. (1989) Immunological characterization of human phenol sulfotransferase. *Mol. Pharmacol.* **36**, 29–33
31. Li, X., Clemens, D.L., Cole, J.R., and Anderson, R.J. (2001) Characterization of human liver thermostable phenol sulfotransferase (SULT1A1) allozymes with 3, 3′, 5-triiodothyronine as the substrate. *J. Endocrinol.* **171**, 525–532
32. Kester, M.H., Bulduk, S., Tibboel, D., Meinl, W., Glatt, H., Falany, C.N., Coughtrie, M.W., Bergman, A., Safe, S.H., Kuiper, G.G., Schuur, A.G., Brouwer, A., and Visser, T.J. (2001) Potent inhibition of estrogen sulfotransferase by hydroxylated PCB metabolites: a novel pathway explaining the estrogenic activity of PCBs. *Endocrinology* **141**, 1897–1900
33. Yager, J.D. (2000) Endogenous estrogens as carcinogens through metabolic activation. *J. Natl Cancer Inst. Monogr.* **27**, 67–73