Sex steroid hormones regulate constitutive expression of Cyp2e1 in female mouse liver

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CYP2E1 is involved in xenobiotic-induced toxicity and carcinogenicity. It catalyzes the metabolism and bioactivation of a broad variety of low-molecular-weight (<100) and hydrophobic agents, including procarcinogens and solvents, and metabolizes drugs, such as isoniazid, chlorozoxazone, coumarin derivatives, gas anesthetics, and acetaminophen, with potential hepatotoxic and nephrotoxic properties (3, 18, 22–25, 36, 56, 79, 81). It is also worth noting that nitrosamines are metabolized by CYP2E1 to carcinogenic metabolites (81). Arachidonic acid and its metabolites that are lipid second messengers involved in cellular signaling and inflammation (4) are also substrates of CYP2E1 (15).

It should be also underscored that, in several pathophysiological states such as diabetes, obesity, and fasting, Cyp2e1 expression was detected at higher levels in both experimental animals and humans compared with normal individuals, and this increase was attributed to increased ketone body levels present in these pathologies (6, 16, 17, 22, 32, 55, 56, 63, 64, 77, 82). The determinant contribution of CYP2E1 in oxidative stress should be also added to the broad array of biological roles this cytochrome holds. Reactive oxygen species liberated during CYP2E1-catalyzed xenobiotic metabolism can trigger mitochondrial damage, DNA modification, lipid peroxidation, cytokine production, and even cell death (9, 10, 22). In addition, a novel metabolic pathway of estrogens involves CYP2E1. This CYP along with CYP1A1 and CYP2B6, is involved in estrone and estradiol conversion to quinol metabolites (50).

The multifactorial differentiation in the biological profile of males and females including drug metabolizing systems, added to the cross-talk between the steroid receptor-linked signaling pathways and those pathways regulating CYP2E1, set the necessity for further investigation of the sex-specific differences and the role of female sex steroid hormones in CYP2E1 regulation (59). Since sex steroid hormones are the basis of the widely used contraceptives and hormonal replacement therapy in menopausal women for the prevention of osteoporosis and...
cardiovascular events (26, 58), this study investigated the role of female sex steroid hormones in hepatic Cyp2e1 regulation, using ovariec-tomized mice supplemented with 17β-estradiol and/or progesterone. The role of estrogens was also evaluated in intact cyclic females treated with tamoxifen, a drug with antiestrogenic effects in the breast tissue that is used as standard endocrine treatment in women with hormone receptor-positive breast cancer. Tamoxifen, though, under certain circumstances, can also exert estrogenic agonist properties depending on the tissue (46). In addition, the hepatic Cyp2e1 expression pattern was assessed at the four distinct phases of the estrous cycle of intact cyclic female mice and compared with the male Cyp2e1 expression profile. A marked diversity in hepatic Cyp2e1 expression was observed within the different phases of the estrous cycle, with progesterone holding a critical regulatory role.

MATERIALS AND METHODS

Animals and treatment. Wild-type and CYP2E1-humanized mice, established by insertion of the human CYP2E1 transgene into Cyp2e1-null mice on the C57BL/6J background (12), were housed in groups of three to five in plastic cages in a temperature- and light-controlled environment. Standard rodent chow and tap water were provided ad libitum. Animals were adapted to handling for an adaptation period of 1 wk prior to the experiment. All procedures were carried out in accordance with Institute of Laboratory Animal Resources guidelines and approved by the National Cancer Institute Animal Care and Use Committee.

Estrous cycle monitoring. Intact cyclic female 6-wk-old wild-type mice were screened for their estrous cycle integrity for 2 wk. Only female mice with a normal estrous cycle were included in this study. They were divided into four groups according to the phase of the estrous cycle on the last day of the experiment. Intact male mice of the same age were also included. Estrous cycle phase was monitored by analysis of the cell types in vaginal lavages collected from all cyclic female mice. Smears were obtained daily between 1100 and 1300 h for at least 15 consecutive days. The fire-polished and shortened tip of a Pasteur pipette carrying one drop of tap water was placed at the vaginal orifice, and care was taken not to insert it more than 1 mm to minimize the possibility of cervical stimulation and thus disruption of the cyclicity of the estrous cycle (48). Vaginal smears were spread gently on a microscope slide and allowed to dry. Slides were then fixed with absolute methanol (3 min), drained, and stained with Giemsa solution (2%, Merck) for at least 20 min. Staining is essential for the accurate identification of the cycle stage: cytoplasm stains blue and nuclei stain red. Identification of cell types was made microscopically according to published methods (83). Proestrus (PE), estrus (E), metestrus (ME), and diestrus (DE) are the four distinct phases of the estrous cycle. Persistent DE or E and cycles lasted longer than 5 days were considered abnormal, and animals demonstrating abnormal estrous cycles were excluded.

Ovariectomy and hormonal supplementation. Intact cyclic female 5-wk-old wild-type mice (C57BL/6J) were bilaterally ovariectomized under gas anesthesia and implanted with pellets containing either 17β-estradiol or progesterone (3-wk release 17β-estradiol or progesterone pellets; Innovative Research of America, Sarasota, FL). Steroid hormone containing pellets released either ~25 μg of 17β-estradiol or 425 μg of progesterone per day, levels close to those observed in intact cyclic female mice (48). The operation lasted 15–20 min, and the animals were returned to their cages after recovery, where they remained until the end of the experiment, 21 days later. Sham-operated cyclic female rats and those implanted with a placebo pellet at estrus were used as controls.

Anesthesia technique. Anesthesia was performed using isoflurane (IsoFlo, Abbott Park, IL) in spontaneously breathing mice through a vaporizer providing standardized gas concentration to an outlet tube. Isoflurane was administered at a constant flow rate of 1.0–2.0 l/min with oxygen at a concentration of 30–35%. The animals were placed in an anesthesia induction box of 20 cm diameter and 10 cm height. A silicon tube providing the anesthetic gas mixture was connected. Anesthesia was induced by inhalation of 4% vaporized isoflurane. This led to a rapid induction of anesthesia within 15–30 s, allowing the animals to be placed in a prone position. It also allowed the type of inhalation to be changed to a tube of about 1.5 cm diameter surrounding the head and to reduce the isoflurane vaporization to 3.0–3.5%. This concentration provided deep anesthesia, allowing the surgical procedure to be performed without any clinical sign of pain or changes of macrohemodynamic parameters [mean arterial pressure (MAP) and heart rate (HR)]. This consisted of the following: no movements of the animal, no reaction to pain stimuli during surgical procedures (foot pad reaction), and stable blood pressure, HR, and respiratory rate. Under these conditions, anesthesia was maintained until the end of the operation. Buprenorphine HCl (0.25 mg/kg sc; Bedford Labs) was given after the final incision closure, and the animal was placed on a heating pad and allowed to recover before returning to its home cage. Locomotor activity, daily food and water consumption, and body weight progression showed no abnormalities 24 h after anesthesia.

Tamoxifen treatment. Intact cyclic 8-wk-old wild-type C57BL/6J female mice were treated with tamoxifen (2 mg/kg ip) for 3 days. The mice were killed 24 h after the last dose. Controls received normal saline for 3 days and were euthanized at estrus. All mice used in this study were euthanized by carbon oxide asphyxiation.

Assessment of hepatic p-nitrophenol hydroxylase activity. Microsomal fractions were prepared from parts of the liver dissected from individual mice by applying differential centrifugation (39). Microsomal protein content was determined by the method of Lowry et al. (42). For enzyme activity, CYP2E1-dependent p-nitrophenol hydroxylase (PNP) activity was determined spectrophotometrically by measuring the concentration of 4-nitrocatechol in 1 mg/ml microsomal protein. This compound is the metabolic product of p-nitrophenol, which was used as a substrate for CYP2E1 (53).

Quantitative real-time PCR. For the isolation of total RNA from livers, TRIzol reagent (Invitrogen, Carlsbad, CA) was used following the manufacturer’s protocol. The concentration of total RNA was determined spectrophotometrically. Quantitative real-time reverse transcriptase PCR (qPCR) was performed with cDNA generated from 1 μg of total RNA with a SuperScript III reverse transcriptase kit (Invitrogen). Gene-specific primers were designed for qPCR using the Primer Express software (Applied Biosystems, Foster City, CA). The sequences for the forward and reverse primers used are shown in Table 1. SYBR Green PCR Master Mix (Applied Biosystems, Warrington, UK) was used for the real-time reactions, which were carried out using the ABI Prism 7900 HT sequence detection system (Applied Biosystems). The PCR conditions were the following: 95°C for 10 min followed by 40 cycles of 95°C for 15 s, 60°C for 1 min, and finally one cycle of 95°C for 15 s, 60°C for 15 s, and 95°C for 15 s. Relative mRNA expression levels were normalized to β-actin, and values were quantified using the comparative threshold cycle method.

Western blot analysis. Immunoblot analyses of CYP2E1, total and phosphorylated-Akt, JNK, STAT5b, and FOXO1a apoprotein were carried out using microsomes, total cellular proteins, cytosol, or nuclear extracts of liver samples, respectively. RIPA buffer supplemented with protease inhibitors, PMSF (10 μM), BGP (50 μM), and NaF (50 μM) was used for the extraction of liver total cellular proteins. For the preparation of the nuclear extracts and cytosol, the NE-PER nuclear extraction kit (Pierce, Rockford, IL) was used. Protein concentration was determined in the samples using the BCA protein assay method (Pierce). Proteins were subjected to sodium dodecyl sulfate-polyacrylamide (7%) gel electrophoresis and immunoblotting using the following antibodies: mouse monoclonal CYP2E1 antibody (Cell Signaling Technology), rabbit monoclonal

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was used as loading control. Secondary anti-rabbit or anti-mouse antibodies conjugated to horseradish peroxidase (Santa Cruz Biotechnology) were used, and the proteins were detected using a chemiluminescence detection kit (ECL, Amersham, GE Healthcare).

Hormonal determinations. 17β-Estradiol, the major estrogen secreted by premenopausal ovaries, and progesterone concentrations were determined in the plasma of intact and tamoxifen-treated female mice at estrus and in those mice subjected to bilateral ovariectomy and receiving hormonal replacement therapy. For determinations of estradiol and progesterone levels, an EIA corresponding kit (Cayman Chemicals) was used.

Statistical analysis. The data are presented as means ± SE and were analyzed using two-way ANOVA followed by multiple comparisons with Bonferroni’s significant difference method. The significance level for all analyses was set at a probability of ≤0.05. Moreover, correlation statistical analysis was performed using Pearson’s coefficient correlations to investigate possible correlations between alterations in the relative Cyp2e1 mRNA expression and those observed in several key regulators of this CYP, such as hepatocyte nuclear factor (Hnf)-1α, β-catenin, and the sterol-regulatory element-binding protein-1c (Srebp-1c) following ovariectomy or hormonal replacement.

RESULTS

Assessment of sex differentiation in Cyp2e1 expression. The expression profile of Cyp2e1 mRNA was evaluated in the liver of male and cyclic female mice. The hepatic expression of
Cyp2e1 mRNA, protein, and activity levels were found to fluctuate within the different phases of the estrous cycle of female mice with higher expression at E and markedly lower expression at ME (Fig. 1A, P < 0.001). Pearson’s coefficient correlation analysis indicated that the hepatic Cyp2e1 expression pattern in the distinct phases of the estrous cycle was highly correlated with the expression pattern of mRNAs encoding Hnf-1α (Fig. 1B, P < 0.001), β-catenin (Fig. 1C, P < 0.001) and Srebp-1c (Fig. 1D, P < 0.001), transcription factors previously found to be involved in the regulation of Cyp2e1. Hepatic Cyp2e1 mRNA, protein, and activity levels were markedly lower in males than in cyclic females at E (Fig. 2A, P < 0.001) and were at equivalent levels with those observed in cyclic females at ME and in ovariectomized mice (Figs. 1A and 2A). These data clearly indicate differentiation in the hepatic Cyp2e1 expression patterns between male and female mice. In addition, the fluctuation of Cyp2e1 expression within the different phases of the estrous cycle in females is also apparent.

Assessment of the role of female sex steroid hormones in Cyp2e1 regulation. Ovariectomy-induced depletion of estrogens and progesterone was followed by a strong suppression of hepatic Cyp2e1 mRNA, protein, and activity levels compared with sham-operated females at E (Fig. 2A, P < 0.001). When ovariectomized mice were supplemented with pellets carrying 17β-estradiol, hepatic Cyp2e1 mRNA levels increased (Fig. 2B, P < 0.001), but this increase was not followed by increased CYP2E1 apoprotein and activity levels (Fig. 2B). When ovariectomized mice were implanted with progesterone pellets, hepatic Cyp2e1 mRNA, protein, and activity levels were increased (Fig. 2B, P < 0.001). Similarly, ovariectomized mice supplemented with both 17β-estradiol and progesterone had increased hepatic Cyp2e1 mRNA, protein, and activity levels (Fig. 2B, P < 0.001) compared with those receiving the placebo treatment. It is of interest to note that alterations in relative Cyp2e1 mRNA expression, which were induced by ovariectomy and/or hormonal replacement using 17β-estradiol and/or progesterone, were correlated with plasma steroid hormone levels (Fig. 2, C and D). These alterations in hepatic Cyp2e1 mRNA expression were also highly correlated with Hnf-1α (Fig. 3A, P < 0.001), β-catenin (Fig. 3B, P < 0.001), and to a lesser extent Srebp-1c (Fig. 3C, P < 0.001) mRNA levels. Pgc-1α mRNA transcripts were also increased by hormonal replacement with either 17β-estradiol and/or progesterone in ovariectomized mice compared with placebo-treated castrated mice (Fig. 4, P < 0.001).

Ovariectomy had no effect on CYP2E1 mRNA levels in the livers of a CYP2E1-humanized mouse model. It should be noted that supplementation with 17β-estradiol repressed hepatic CYP2E1 expression in ovariectomized CYP2E1-humanized mice (Fig. 5, P < 0.01), whereas progesterone supplementation had no effect (Fig. 5).

These data indicate the determinant role of sex steroid hormones in hepatic Cyp2e1 regulation, with the role of progesterone being more prominent than that of estradiol. A phenotype in hepatic CYP2E1 regulation by sex steroid hormones is also apparent between wild-type and CYP2E1-humanized mice.
Assessment of the involvement of major signal transduction pathways in steroid hormone-induced regulation of Cyp2e1. The insulin controlled PI3K/Akt signaling pathway holds a critical role in Cyp2e1 regulation (29, 51, 62, 77). To assess the involvement of this signaling pathway in the sex steroid hormone-mediated Cyp2e1 regulation, the phosphorylated state of Akt after ovariectomy was detected following ovariectomy, but it was markedly suppressed in the nucleus of ovariectomized males compared with intact males at E (Fig. 6). In females compared with males (Fig. 8). Tamoxifen markedly increased Akt phosphorylation (Fig. 8).

Based on these findings, it appears that the sex steroid hormone-mediated modification of Cyp2e1 regulation in female mice potentially involves the insulin/PI3K/Akt signaling pathway. Assessment of the role of tamoxifen in Cyp2e1 regulation. Treatment of intact females with tamoxifen reduced Cyp2e1 mRNA transcripts in the liver (Fig. 9A, P < 0.001). A similar effect of tamoxifen was observed on Hnf-1α, β-catenin, and Pgc-1α mRNA levels in females compared with males (Fig. 8). Tamoxifen also strongly increased Akt phosphorylation (Fig. 9B), which in turn stimulated FOXO1 activation (Fig. 9B). The drug also markedly decreased JNK phosphorylation.

**Fig. 3.** Assessment of impact of hormonal replacement on Cyp2e1 expression. Effect of hormonal replacement with E2 and/or progesterone on Cyp2e1 mRNA levels. All comparisons took place with basal mRNA levels in OV mice. Values are expressed as means ± SE (n = 10). **P < 0.01, ***P < 0.001.

**Fig. 4.** Effect of hormonal replacement with E2 and/or progesterone on Pgc-1α mRNA expression. Comparison took place with basal Pgc-1α mRNA levels in OV mice. ***P < 0.001.
Fig. 5. Effect of hormonal replacement with E2 and/or progesterone on CYP2E1 expression in intact mice. CYP2E1 mRNA and activity levels are expressed as means ± SE (n = 10). Comparisons took place with basal CYP2E1 mRNA or PNP activity levels in OV mice, respectively. **P < 0.01. CYP2E1 Western blot images contain 1 sample per treatment. Each lane in the capture represents CYP2E1 protein amount in the microsomes of 1 transgenic mouse.

Although there is no direct evidence, the above data profoundly indicate that tamoxifen repressed Cyp2e1 expression by mechanisms involving activation of the PI3K/Akt/FOXO1 and GH/STAT5b-linked pathways.

Fig. 6. Assessment of hormonal replacement in ovariectomized mice. The levels of 17β-estradiol and progesterone in sham-operated female mice at E were higher than those observed in ovariectomized placebo-treated mice (Table 2, P < 0.05 and P < 0.01, respectively). As expected, implantation of pellets carrying either 17β-estradiol and/or progesterone in bilaterally ovariectomized mice markedly supplemented the corresponding hormonal levels (Table 2, P < 0.001 and P < 0.01, respectively). It is worth noting that plasma progesterone levels in ovariectomized mice implanted with pellets carrying progesterone were equivalent to those detected in females at E (Table 2). It should be noted that plasma 17β-estradiol levels were many fold higher in ovariectomized mice supplemented with estradiol than in intact females at E (Table 2, P < 0.001). Tamoxifen treatment of cyclic females was followed by decreased serum progesterone levels compared with controls at E, whereas serum 17β-estradiol concentration was not significantly affected (Table 2, P < 0.01).

**DISCUSSION**

The multifactorial and complex process of CYP2E1 regulation has been demonstrated by previous studies (22, 29, 37; Fig. 10) indicating that diverse factors may modify CYP2E1 expression at the levels of transcription (2, 9), mRNA stabilization (65, 80), mRNA translation (32), protein synthesis (66), and protein degradation (54, 56). Among these factors, hormones hold a critical role (8, 56). Nonetheless, despite intense investigation, the sex differentiation and the role of female sex steroid hormones in constitutive CYP2E1 regulation remain unclear (59).

Interestingly, the data of the present study revealed differential hepatic Cyp2e1 expression patterns within the distinct phases of the estrous cycle of female mice, with lower expression at methestrus and higher at estrus. Hepatic Cyp2e1 expression was significantly increased in both intact females at estrus and ovariectomized females supplemented with estradiol and progesterone compared with intact females at methestrus and ovariectomized females receiving placebo (Table 2, P < 0.05).

Fig. 7. Effect of hormonal replacement with E2 and/or progesterone on eNos (A) and iNos (B) mRNA expression. Values are expressed as means ± SE (n = 10). All comparisons took place with basal mRNA levels in OV mice. *P < 0.05, **P < 0.01, ***P < 0.001.
pression was markedly lower in males than in females at estrus, whereas depletion of female sex steroid hormones by ovariectomy (hormonal state similar to menopause) brought Cyp2e1 expression to levels observed in males and females at ME. When ovariectomized mice were supplemented with progesterone, Cyp2e1 expression approached that at estrus. Estrogens also increased hepatic Cyp2e1 mRNA expression in ovariectomized mice, whereas tamoxifen, an antiestrogenic agent, markedly repressed the expression of Cyp2e1 in the livers of intact female mice. This finding is in line with that of a previous study reporting that the antiestrogen toremifene alleviated ethanol induction of CYP2E1 (30). It is well established that tamoxifen competitively binds to ERs on tumors and other tissue targets, producing a nuclear complex that inhibits estrogen effects. Specifically, the ER/tamoxifen complex recruits corepressors to stop genes being switched on by estrogens (43, 46), a fact that may explain, at least in part, the suppressive effect of tamoxifen on Cyp2e1 regulation. The tamoxifen-induced repression in Cyp2e1 may also be attributed to the lower serum progesterone levels detected in drug-treated mice compared with controls. This downregulating process potentially involves several major signal transduction pathways, including PI3K/Akt/FOXO1, GH/STAT5b, and related pathways, among others. Nonetheless, further investigation is needed to directly correlate the alterations in the aforementioned pathways with those related with the sex steroids on Cyp2e1.

It is of interest to note that ovariectomy had no effect on CYP2E1 expression in the livers of mice carrying the human gene, whereas 17β-estradiol markedly downregulated CYP2E1 mRNA expression in the livers of intact female mice. This finding is in line with that of a previous study reporting that the antiestrogen toremifene alleviated ethanol induction of CYP2E1 (30). It is well established that tamoxifen competitively binds to ERs on tumors and other tissue targets, producing a nuclear complex that inhibits estrogen effects. Specifically, the ER/tamoxifen complex recruits corepressors to stop genes being switched on by estrogens (43, 46), a fact that may explain, at least in part, the suppressive effect of tamoxifen on Cyp2e1 regulation. The tamoxifen-induced repression in Cyp2e1 may also be attributed to the lower serum progesterone levels detected in drug-treated mice compared with controls. This downregulating process potentially involves several major signal transduction pathways, including PI3K/Akt/FOXO1, GH/STAT5b, and related pathways, among others. Nonetheless, further investigation is needed to directly correlate the alterations in the aforementioned pathways with those related with the sex steroids on Cyp2e1.

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expression in the ovariectomized CYP2E1-humanized mice. Progesterone alone had no effect, but it blocked the downregulating effect of 17β-estradiol on CYP2E1 when castrated mice were treated with both hormones. This effect is potentially associated with the fact that progesterone acts as an antagonist of estradiol on ER gene expression (78). These differential effects on hepatic Cyp2e1 regulation, which were observed in wild-type C57BL/6J and CYP2E1-humanized mice, revealed the existence of a phenotype in the regulation of CYP2E1 expression by female sex steroid hormones. However, this experimental setting could not be used to pinpoint any differential CYP2E1 expression between rodents and humans.

It is well documented that Cyp2e1 expression is controlled by the liver-enriched homeodomain-containing transcription factor Hnf-1α (2). Nonetheless, recent reports revealed that factors other than Hnf-1α are also critical in the regulation of Cyp2e1, including β-catenin (52, 60, 68), which potentially modifies the expression of a transcriptional coactivator essential for Cyp2e1 activation by Hnf-1α or a micro-RNA destabilizing Cyp2e1 mRNA. It is also possible that β-catenin modifies Cyp2e1 expression at the translation level (22, 40). The data of the present study suggest a critical role for these two factors in the regulation of Cyp2e1, as all differences observed in the expression pattern of this CYP, those related to sex, ovariectomy, sex steroid hormones, or tamoxifen were highly correlated with Hnf-1α and β-catenin mRNA expression. It is possible that β-catenin, the main effector of the Wnt pathway, acts as a potent transcriptional coactivator of androgen (AR), estrogen (ER) and progesterone (PR) receptors (38, 47). This hypothesis is supported by a previous study using immunoprecipitation (ChIP) analysis, which revealed that Wnt and estrogen signaling pathways cross-talk in vivo through functional interaction between EREs and β-catenin (38, 47).

Insulin signaling holds a critical role in the transcriptional and posttranscriptional regulation of CYP2E1. In particular, the insulin-induced downregulation of CYP2E1 suggests involvement of phosphatidylinositol 3-kinase (PI3K) and a variety of downstream enzymes. In this regulatory pathway, a major intermediate effector is the PI3K-activated serine/threonine kinase Akt/PKB (51, 77). The present findings revealed that ovariectomy increased Akt phosphorylation, whereas supplementation with progesterone and/or estrogens brought phosphorylation levels back to those detected in females at estrus. Akt phosphorylation was also higher in males than in females at estrus and tamoxifen strongly increased it. These findings indicate that cross-talk between the sex steroid hormone recep-

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Table 2. Plasma steroid hormone levels in C57BL/6J female mice

<table>
<thead>
<tr>
<th>Treatment</th>
<th>17β-Estradiol</th>
<th>Progesterone</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham at Estrus</td>
<td>6.4 ± 0.6*</td>
<td>5.151 ± 888.6†</td>
</tr>
<tr>
<td>Ovariectomized Placebo</td>
<td>3.9 ± 0.2</td>
<td>554.4 ± 79.5</td>
</tr>
<tr>
<td>17β-Estradiol</td>
<td>88.7 ± 14.6‡</td>
<td>3.019.1 ± 866.2†</td>
</tr>
<tr>
<td>Progesterone</td>
<td>7.7 ± 2.0</td>
<td>3.511.6 ± 361.5‡</td>
</tr>
<tr>
<td>17β-Estradiol + progesterone</td>
<td>56.0 ± 8.3‡</td>
<td>5.607.3 ± 379.8‡</td>
</tr>
<tr>
<td>Control at Estrus</td>
<td>11.7 ± 3.5</td>
<td>4.155.0 ± 262.0</td>
</tr>
<tr>
<td>Tamoxifen</td>
<td>16.8 ± 5.8</td>
<td>1.697.5 ± 111.7‡</td>
</tr>
</tbody>
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Values are expressed as means ± SE in pg/ml (n=10). Assessment of the effect of castration and hormonal replacement on plasma sex steroid levels. Plasma sex steroid hormone concentration in C57BL/6J female mice at estrus or following ovariectomy and hormonal replacement with 17β-estradiol and/or progesterone. *P < 0.05, †P < 0.01, ‡P < 0.001.
tors and insulin/PI3K/Akt signaling potentially exists (58) that may be involved in the regulation of Cyp2e1. It appears that predominantly progesterone inactivates several downstream elements in the PI3K/Akt/FOXO1 signaling pathway, an effect that was blocked by estradiol. In this pathway, SREBP-1c regulates various genes including CYP2E1 (1, 35, 84). Srebp-1c mRNA levels correlated with the fluctuations of Cyp2e1 mRNA expression in the distinct phases of the estrous cycle and the changes induced by ovariectomy, sex steroid hormones, and tamoxifen.

GH is a major endocrine factor modulating Cyp2e1 expression in the liver (11, 28, 42, 71, 76, 77, 80), with a determinant role in the sexual dimorphism observed in the expression of numerous genes (45, 74, 76). GH binds to the GH receptor on the cell membrane and activates signaling pathways including that by the GH pulse-activated transcription factor STAT5b (27), which is essential for the liver sexual dimorphism (14). The present study confirmed that, in the female liver, STAT5b activation is generally low compared with that in males (13) and is potentially connected, at least in part, with the lower Cyp2e1 expression levels detected in the male liver compared with that in intact females at E.

In the wide array of factors interfering in the regulation of various CYPs, NO, an important inflammatory mediator synthesized by both NO synthases, eNOS and iNOS, is recognized as an important regulator. However, in the present experimental setting, alterations in hepatic eNOS and iNOS mRNA expression followed ovariectomy or hormonal replacement, repression, and upregulation, respectively, appear not to be connected with those in Cyp2e1 expression, as both NOS isoforms are connected with CYP downregulation (21, 31, 44).

It is possible that transcription factors other than those mentioned above play critical roles as determinants of sexual dimorphism in Cyp2e1 regulation, and their actions are probably influenced by sex steroid hormones.

Taken together, the above data clearly indicated a role for female sex steroid hormones in the upregulation of Cyp2e1 expression in the liver of female mice. This effect is potentially mediated by inactivation of the insulin/PI3K/Akt signaling pathway. This study also confirmed a sex differentiation in the hepatic expression of Cyp2e1, with higher levels detected in intact cyclic females at E and lower in males at levels close to those detected in estrogen- and progesterone-depleted female mice (ovariectomized) and in cyclic females at ME. Notably, variation in the expression patterns of Cyp2e1 within the distinct phases of the estrous cycle is highly correlated with the fluctuations in the levels of gonadal hormones in plasma, 17β-estradiol and progesterone. On the basis of these findings and previous reports (73, 75), the present study suggests that differentiation in hepatic Cyp2e1 expression pattern between females and males is potentially mediated by the GH/STAT5b-related signaling pathway. The present data contribute to a better understanding of the selectivity that governs the CYP2E1-induced transformation of molecules in females and males. This knowledge will allow more successful predictions in pharmaco- and toxicokinetic properties of the CYP2E1 substrates, thus providing insights exploitable in novel drug development and assessment of risk associated with exposure to drugs, environmental chemicals, and procarcinogens. It also provides information about potential perturbations of endogenous regulatory circuits with associated pathophysiological consequences (72).

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

Author contributions: M.K. conception and design of research; M.K. and J.C. performed experiments; M.K. and F.J.G. interpreted results of experiments; M.K. prepared figures; M.K., J.C., and F.J.G. drafted manuscript; M.K., J.C., and F.J.G. approved final version of manuscript.

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