Estrogen Receptor Reduces CYP1A1 Induction in Cultured Human Endometrial Cells*

M. Stacey Riccić, Diane G. Toscano, Carolyn J. Mattingly, and William A. Toscano, Jr.§

From the Department of Environmental Health Sciences and Center for Bioenvironmental Research, Tulane University School of Public Health and Tropical Medicine, New Orleans, Louisiana 70112-2699

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2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) exerts its toxic action via the aryl hydrocarbon (Ah) receptor, which induces a battery of xenobiotic-metabolizing enzymes, including the cytochrome P450 isozyme, CYP1A1. TCDD-induced 7-ethoxycoumarin-O-deethylase activity was reduced 75% in cultured human endometrial ECC-1 cells exposed to various concentrations of 17b-estradiol for up to 72 h, with a half-maximal effective concentration (EC50) of 0.9 nm. Reduced enzyme activity was correlated with decreased CYP1A1 mRNA levels, and transcription. Exposure to TCDD plus 17b-estradiol also reduced CYP1A1 activity in MCF-7 breast cancer cells but not in Hep-3B human liver cells or HuE primary human keratinocytes, suggesting that the effect was specific to estrogen-regulated cells. Estrogen receptor antagonists 4-hydroxytamoxifen and 7α-(4,4,5,5,5-pentafluoro-pentylsulfinyl)monoyl estr-1,3,5(10)-triene3, 17b-diol restored TCDD-induced CYP1A1 transcription, steady-state mRNA levels, and enzymatic activity in ECC-1 cells. Gel mobility shift assay showed that 17b-estradiol had little effect on Ah receptor binding to its DNA-responsive element. 17b-Estradiol did not alter the induction of another Ah receptor-regulated gene, CYP1B1, suggesting that altered Ah receptor binding to DNA does not mediate reduced CYP1A1 transcription. Transfecting ECC-1 cells with a general transcription factor involved in CYP1A1 induction, nuclear factor-1, reversed 17b-estradiol antagonism of dioxin-induced CYP1A1. The data suggest that 17b-estradiol reduced CYP1A1 expression at the transcriptional level by squelching available nuclear factor-1, a transcription factor that interacts with both Ah and estrogen receptors.

TCDD, also known as dioxin, is the archetype of a family of related polychlorinated compounds found ubiquitously in the environment. Exposure of animals to TCDD results in many toxic actions (see Ref. 1 for a review). Dramatic differences in dioxin toxicity have been observed between the sexes of some animal species, suggesting hormonal modulation of dioxin action (2).

Dioxins are lipophilic compounds that readily pass through membranes and bind to an intracellular receptor with no known enzymatic function, called the Ah receptor (for a review, see Ref. 3). The Ah receptor is a ligand-activated transcription factor that stimulates gene expression when coupled with another structurally related protein, Ah receptor nuclear translocator (4). The heterodimer binds specific DNA sequences, AhREs, and initiates transcription of various genes, including those for xenobiotic metabolizing enzymes (5).

Estrogen regulates the proliferative cycle of the endometrium and mammary glands by binding to estrogen receptor and stimulating transcription, in part, by inducing growth factors and growth factor receptors (for a review, see Ref. 6). Like the Ah receptor, estrogen receptor is a transactivating enhancer protein. Upon ligand binding, estrogen receptor forms a homodimer that recognizes specific DNA sequences, estrogen response elements, located in target genes. Estrogen-responsive tissues are particularly sensitive to dioxin actions, some of which are not toxic. For example, TCDD exerts protective effects against the appearance of benign mammary and uterine tumors in Sprague-Dawley rats (7). An epidemiology study showed that breast and endometrial cancers were slightly reduced in women exposed to dioxin as a result of an industrial accident that occurred in Seveso, Italy (8). In contrast, rhesus monkeys exposed to TCDD showed a concentration-dependent increase in the incidence and severity of endometriosis (9), a painful condition correlated with infertility (10). The association of endometriosis with TCDD exposure has resulted in several medical hypotheses linking its incidence in humans with environmental exposure to dioxin (11, 12).

A mechanism explaining the interaction between estrogen and dioxin signaling has yet to be defined. Some investigators have suggested that TCDD is anti-estrogenic (13) because it antagonizes many estrogen actions, including estrogen-stimulated proliferation of cultured mammary cells and estrogen-stimulated increase in uterine weights (for a review, see Ref. 14). TCDD is not a typical estrogen antagonist, however, because it does not compete with estrogen binding to its receptor (15). Instead, TCDD induces three known cytochrome P450 isozymes that hydroxylate 17b-estradiol, the most biologically potent estrogen, to various catechols (16). These isozymes, CYP1A1, CYP1A2, and CYP1B1, are under direct transcriptional regulation by Ah receptor interacting at AhREs (17–19). These cytochromes play important roles in xenobiotic metabolism but also appear to mediate 17b-estradiol hydroxylation in some cells.

Conversely, estrogen appears to mediate Ah receptor-mediated cytochrome P450 monooxygenase activity in many differen
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ENT systems. This was first observed by Nebert et al. (20) almost 30 years ago, when reduced cytochrome P450-catalyzed aryl hydrocarbon hydroxylase activity in both animals and cultured cells was observed after exposure to 17β-estradiol. This observation has appeared periodically in the literature, but no mechanism has unequivocally defined the effect (21–25).

In this study, we used an estrogen-responsive clonal endometrial epithelial cell line, ECC-1, to examine the mechanism by which estrogen receptor modulates dioxin-responsive genes. We chose ECC-1 cells as our model system because they contain functional estrogen and Ah receptors (26, 27). We report that TCDD-induced CYP1A1 was diminished at the transcriptional level when ECC-1 cultures were also exposed to 17β-estradiol. We present data showing restoration of dioxin-induced CYP1A1 message and activity in cultures exposed to two estrogen receptor antagonists. We also show that reduction of CYP1A1 activity after exposure to 17β-estradiol was specific to estrogen-regulated cells. We present gel mobility shift data showing no reduction of Ah receptor binding to AhRE and demonstrate reversal of 17β-estradiol-mediated reduction of TCDD-induced transcription by transient transfection of NF-1, a general transcription factor involved in CYP1A1 induction.

EXPERIMENTAL PROCEDURES

Materials—TCDD was obtained from Cambridge Isotopes Laboratory (Andover, MA). Dr. A. Wakeling (Zeneca Pharmaceuticals) kindly provided ICI 182,780. The restriction enzymes Rsal, HindIII, PvuII, SacI, PstI, PvuII, SacI, Xhol, Bsp EI, NheI, and Klenov fragment (3′–5′ exonuclease and T4 DNA ligase were purchased from New England Biolabs, Beverly, MA. Restriction enzymes BgII and BamHI and T7 RNA polymerase, DNA polymerase I large Klenov fragment, and pGL3-Basic vector were from Promega (Madison, WI), and Klenov fragment (3′–5′ exonuclease) and T4 DNA ligase were purchased from New England Biolabs, Beverly, MA. Restriction enzymes BgII and BamHI and T7 RNA polymerase, DNA polymerase I large Klenov fragment, and pGL3-Basic vector were from Promega (Madison, WI).

We used cultured primary keratinocytes derived from donor foreskin (28). Hesp-3B (ATCC HB-8064) human liver cells were from Dr. Ali Scandurro, Tulane Medical School. Dr. Louise Nutter (University of Minnesota, Minneapolis, MN) provided MCF-7 breast cells. For culturing ECC-1 cells, an in vitro method developed for dermal epithelium (28) was adapted to model the morphology of the endometrial epithelium. ECC-1 cells were grown on a layer of lethally irradiated newborn NIH 3T3 cells, which mimics the normalcy and enhances attachment. ECC-1 cells grown in this manner typically reached confluence after 8 days. ECC-1, MCF-7, and HuE cells were all cultured in Dulbecco’s modified Eagle’s medium (Life Technologies, Inc.), containing 5% iron-supplemented bovine calf serum (Hyclone, Salt Lake City, UT); ECC-1 cell medium contained 1 ng insulin (Sigma). Cells were grown in an atmosphere of 5% CO2/95% air under saturating humidity at 37 °C. Prior to chemical exposure, cells were grown in 5% charcoal-stripped calf serum in phenol red-free Dulbecco’s modified Eagle’s medium containing 1 ng insulin for a minimum of 5 days. This procedure was necessary to eliminate steroids normally found in serum (29). When cultures reached confluence, they were rinsed with Dulbecco’s modified Eagle’s medium and exposed to either TCDD, 17β-estradiol, or their analogs, dissolved in MeSO unless otherwise stated. MeSO never exceeded 0.1% in the culture medium.

Protein Assay—Protein was estimated using the procedure of Lowry as modified by Peterson (30). Bovine serum albumin was used as a standard. Briefly, cells were rinsed with phosphate-buffered saline and removed from 100-mm culture dishes by scraping with a rubber policeman. Cells were harvested by centrifugation at 500 × g for 3 min, suspended in 10 mM Tris-HCl (pH 7.5), and broken using a Dounce homogenizer. The broken cell suspension was collected by centrifugation (150 × g at 4 °C for 10 min). The supernatant containing the microsomal fraction (100 μl) was added to a reaction mixture containing 32.5 μmol of potassium phosphate, pH 7.2, 250 nmol each of NADPH and NADH, 2.4 μmol of MgCl2, and 250 nmol of 7-ethoxycoumarin (Aldrich) in a total volume of 1 ml. The reaction mixture was incubated for 45 min with shaking at 37 °C. The reaction was terminated by the addition of 125 μl of 15% (v/v) trichloroacetic acid. 7-Hydroxycoumarin formed by the catalytic degradation of the reaction was extracted into chloroform/cyclohexane (3:2) by centrifugation to break emulsions (1000 × g for 5 min). 7-Hydroxycoumarin from the organic phase was extracted using 2 ml of alkaline salt solution (1 N NaCl, 0.01 N NaOH). 7-Hydroxycoumarin in the aqueous phase was measured fluorometrically with a Shimadzu spectrophotofluorometer RF-5301PC (Tokyo, Japan) (λex = 368 nm; λem = 405 nm). For each sample, the concentration was estimated using a standard curve generated from known quantities of 7-hydroxycoumarin. Specific activity was expressed as pmol of 7-hydroxycoumarin formed/mg of protein/min. Assays were carried out under conditions where 7-hydroxycoumarin formation was linear with respect to protein and incubation time.

Radio-labeling of Antisense RNA Probes for Northern Blot Analysis—A plasmid containing the human CYP1A1 gene (pBS1A1) was a generous gift of Dr. Robert Tukey (Cancer Genetics Program, University of California, San Diego, CA) (32, 33). A 1.6-kilobase CYP1A1 DNA fragment containing the T7 RNA polymerase promoter was removed from pBS1A1 by digesting with Rsal restriction enzyme. This fragment produced a 586-base pair antisense ribonucleotide probe (riboprobe) when placed in an in vitro transcription reaction with T7 RNA polymerase (34). The 36B4 cDNA minigene, a generous gift of Dr. Gary Fisher (University of Michigan, Ann Arbor, MI), was used as the loading control because it is not regulated by 17β estradiol (34). A 2.3-kilobase 36B4 DNA fragment containing the T7 RNA polymerase promoter was removed from p36B4 by digesting with Rsal. This fragment produced a 250-base pair riboprobe when transcribed with T7 RNA polymerase. Riboprophes were synthesized by incubating 1 μg of linearized DNA template at 37 °C for 1 h with 4 μl of 5 × transcription buffer (40 mM Tris-HCl, pH 7.5; 6 mM MgCl2; 2 mM spermidine; 10 mM NaCl); 2 μl 100 mM DTT; 20 units of ribonuclease inhibitor (RNAsin, Promega); ATP, GTP, and CTP (2.5 mM each); 100 μM UTP; 50 μCi [α-32P]UTP (specific activity of 600 Ci/mmol); 20 units of T7 RNA polymerase; and nuclease free water to a final volume of 20 μl. The labeled RNA fragments were purified from unincorporated [α-32P]UTP by mini-column chromatography using Bio-Gel P60 (Bio-Rad) and used in hybridization procedures.

Radio-labeling of cDNA for Northern Blot Analysis—The pBS1A1 plasmid was digested with HindIII to obtain a 1.5-kilobase fragment of human CYP1A1 cDNA. A plasmid containing the human CYP1B1 gene (pCYP1B1) was a generous gift of Dr. Thomas Sutter (The Johns Hopkins University) (18). A 1.3-kilobase CYP1B1 cDNA fragment was removed from pCYP1B1 by digestion with PstI and SacI. The p36B4 plasmid was digested with PstI to remove a 760-base pair fragment of human ribosomal phosphoprotein PO cDNA. The fragments were labeled using a published procedure for random oligonucleotide primed DNA synthesis (35). The DNA fragments were denatured, and the DNA was annealed to T7 RNA polymerase promoters for 3 min and immediately submerging into ice. A mixture containing final concentration 0.05 mM dATP/dGTP/dTTP, 50 μCi [α-32P]dCTP (SA = 3000 Ci/mmol), 20 units of Klenov fragment (3′–5′ exonuclease), 10 mM Tris-HCl, pH 7.5, 5 mM MgCl2, and 5 mM DTT was added to the DNA, and the fill-in reaction was carried out for 2 h at 37 °C. The labeled DNA fragments were purified from unincorporated [α-32P]dCTP by mini-column chromatography using Bio-Gel P60 (Bio-Rad).

Northern Blot Analysis—Total RNA was isolated using Tri Reagent® RNA isolation reagent (Molecular Research Center, Inc., Cincinnati, OH). Aliquots of total RNA were separated electrophoretically on denaturing 1% agarose/6 M formamide gels and stained with ethidium bromide to examine the quality and quantity of RNA. Gels were equilibrated in 20 × SSPE (3 M NaCl, 200 mM sodium phosphate, 20 mM EDTA, pH 7.4) and RNA was transferred onto Zeta Probe membranes (Bio-Rad) by capillary action. RNA was cross-linked to membranes with UV radiation using a GS-Genelinker (Bio-Rad) and prehybridized for 1 h in a solution containing 50% formamide, 5 × SSPE, 1% SDS; sonicated, boiled herring sperm (10 μg/ml); and 5 × Denhardt’s solution (0.1% each of bovine serum albumin, polyvinylpyrrolidone, and Ficoll 400). Membranes were hybridized in 50% formamide, 2 × SSPE, 5% dextran sulfate, and enhanced emulsion. Membranes were washed using standard procedures (37) and exposed to Kodak Biomax™ MS double emulsion film at −70 °C for 1–2 days. Autoradiographs were scanned using a 670 imaging densitometer (Bio-Rad), and densitometric volumes of the mRNA bands were calculated using Molecular Analyst™ software (Bio-Rad).

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Plasmid Construction and Transient Transfections—A plasmid containing 7600 base pairs of the 5'-untranslated region of the human CYP1A1 cDNA (the generous gift of Dr. Y. Fuji-Kuriyama) was digested with PvuII to obtain a 1904 base pair fragment (−162/−292) and ligated into the Smal site of the BluescriptSK+ vector (Stratagene, La Jolla, CA). To convert the plasmid to a pCMV-NF1 expression plasmid, the 5'-untranslated region of the NF-1 gene was enzymolitically cloned into NF-1 on CYP1A1 transcription, cells were transfected with an expression plasmid containing the cDNA for NF-1 under transcriptional control of the cytomegalovirus promoter (pCMV-NF1). Dr. Gordon Hager (National Institutes of Health, Bethesda, MD) generously supplied a plasmid vector containing the cDNA for NF-1 under transcriptional control of the cytomegalovirus promoter (pCMV-NF1). The ends were filled with the BglII and BamHI restriction enzymes. The ends were filled with the BglII and BamHI restriction enzymes. This portion of the 5'-untranslated region contains the promoter, exons 1 and 2, and at least three functional AhREs (32, 39). The AhR-responsive fragment was removed by digestion with ScaI and XhoI and inserted into the pGL3-Basic vector upstream of the cDNA encoding firefly luciferase. This plasmid, pGL3–5′-NF1, was used to examine Ah receptor function by transiently transfecting it into ECC-1 cells. In experiments examining the action of NF-1 on CYP1A1 transcription, cells were transfected with an expression plasmid containing the cDNA for NF-1 under transcriptional control of the cytomegalovirus promoter (pCMV-NF1). Ah receptors and respond to TCDD, which induces CYP1A1 activity in both a time- and concentration-dependent manner (27).

RESULTS

Ligand-bound Ah receptor mediates induction of cytochrome P450 monoxygenase(s) by increasing the rate of transcription of these genes (44). Cytochrome P450-catalyzed O-dealkylation of 7-ethoxycoumarin was used to assess induction of enzymatic activity by TCDD (45). ECOD activity is specific for CYP1A1, one of three known cytochrome P450 isozymes induced by TCDD (46). ECC-1 endometrial cells contain functional Ah receptors and respond to TCDD, which induces CYP1A1 activity in both a time- and concentration-dependent manner (27).

Estrogen Reduced CYP1A1 Activity—We examined whether 17β-estradiol altered TCDD-mediated induction of CYP1A1 activity. ECOD activity was measured in cell extracts from ECC-1 cultures exposed to saturating levels of TCDD (10 nM) and various concentrations of 17β-estradiol. Estrogen reduced CYP1A1 activity in a concentration-dependent manner (Fig. 1A). The EC50 for 17β-estradiol modulation of ECOD activity was 0.9 nM, which correlates well with the apparent KD for 17β-estradiol in ECC-1 cells (0.7 nM) (27). CYP1A1 activity in the presence of 17β-estradiol was reduced by 75% compared with that in cells exposed to TCDD alone. The time dependence of 17β-estradiol action was also examined. Exposing ECC-1 cultures to 17β-estradiol resulted in a decrease in ECOD activity from the earliest indication of activity (2 h) that lasted until the experiment was terminated at 65 h (Fig. 1B). Maximal reduction of CYP1A1 activity after exposing ECC-1 cultures to 17β-estradiol in the presence of TCDD occurred after 8 h of exposure and was maintained throughout the time course analysis.

Estrogen Action Was Specific—Exposing ECC-1 cultures to other steroid hormones did not mediate a decrease in TCDD-induced ECOD activity (Fig. 2). Cultures exposed to TCDD plus 4-androstene-3,17-dione, an estrogen precursor, had no effect on the level of induced CYP1A1. Cultures exposed to TCDD plus dexamethasone, a glucocorticoid receptor ligand, and cultures exposed to TCDD plus progesterone, a progesterone receptor ligand, showed a 20% increase in induced ECOD activity. Both the glucocorticoid receptor and the progesterone receptor are present in ECC-1 cultures (27). Glucocorticoids tend to enhance Ah receptor induction of CYP1A1, presumably by glucocorticoid receptor binding to glucocorticoid-responsive elements located within the CYP1A1 gene (47). Progesterone and glucocorticoid receptors bind to the same DNA sequence; therefore, it is possible that the progesterone receptor interacts with the same response element located within the CYP1A1 gene (48). These data suggest that the steroid hormone-mediated decrease in Ah receptor activity was estrogen-specific.

Reversibility of 17β-Estradiol Action—To further establish involvement of the estrogen receptor, we examined whether estrogen receptor antagonists could reverse 17β-estradiol-mediated reduction of CYP1A1 induction. Two structurally different “anti-estrogens,” 4-hydroxytamoxifen and ICI 182,780, after 17β-estradiol binding to estrogen receptor and were used in these experiments to block the estrogen receptor action.
experiments (49, 50). ECC-1 cultures were exposed to combinations of TCDD, \(17\beta\)-estradiol, and either 4-hydroxytamoxifen or ICI 182,780, and ECOD activity was measured after 48 h of exposure. Anti-estrogens reversed estrogen attenuation of TCDD-induced ECOD activity in a concentration-dependent manner (Fig. 3). The EC\(_{50}\) values for 4-hydroxytamoxifen and ICI 182,780 were 750 and 100 nM, respectively. Reversal of estradiol action by estrogen receptor antagonists indicated a role for estrogen receptor in reduction of TCDD induction of CYP1A1 by \(17\beta\)-estradiol.

**Cellular Specificity of \(17\beta\)-Estradiol Action**—To determine whether the observed reduction in CYP1A1 activity was specific to ECC-1 endometrial cells, we examined the effect of \(17\beta\)-estradiol on several human cell lines. We selected MCF-7 mammary carcinoma cells because they, like ECC-1 cells, are derived from estrogen-sensitive tissue and are regulated by \(17\beta\)-estradiol (51–53). As a comparison, we selected Hep-3B human liver cells, and HuE, a primary human keratinocyte cell, neither of which is derived from estrogen-regulated tissues. Similar to ECC-1 cells, \(17\beta\)-estradiol reduced TCDD-induced CYP1A1 activity in MCF-7 cells, but induction of CYP1A1 in Hep-3B and HuE cells by TCDD was unaffected by the presence of \(17\beta\)-estradiol (Fig. 4). To confirm that estrogen

**Fig. 1.** \(17\beta\)-Estradiol decreased TCDD-induced cytochrome P450 activity in a concentration-dependent manner. A, confluent cultures of ECC-1 cells were exposed for 48 h to either 10 nM TCDD alone (CYP1A1 specific activity = 109 ± 11.7 pmol/mg of protein/min) or 10 nM TCDD plus \(17\beta\)-estradiol (\(\widetilde{\alpha}\)) at the concentrations indicated. Cells were harvested and homogenized, and a crude preparation containing the microsomal fraction was assayed for CYP1A1 activity as described under “Experimental Procedures.” Each data point represents the mean from three separate cultures performed in triplicate. B, confluent cultures of ECC-1 cells were exposed to either 10 nM TCDD alone (\(\bullet\)) or TCDD plus \(17\beta\)-estradiol (\(\widetilde{\alpha}\)) (10 nM) for the times indicated. Data are presented as a percentage of the maximum value of CYP1A1 induction (65 h exposure = 73.5 ± 7.5 pmol of 7-hydroxycoumarin formed/mg of protein/min).

**Fig. 2.** Other steroid hormones and steroid receptor agonists do not mediate a decrease in TCDD-induced cytochrome P450 activity. Confluent cultures were exposed for 48 h to 10 nM TCDD plus 10 nM of the chemical indicated (A, 4-androstene-3,17-dione; D, dexamethasone; E, \(17\beta\)-estradiol; P, progesterone). Enzyme activity was assayed as described in Fig. 1. Each column represents the mean from three separate cultures performed in triplicate ± S.E.

**Fig. 3.** Estrogen receptor antagonists reversed \(17\beta\)-estradiol action and reinstated TCDD induction of cytochrome P450 activity. Confluent cultures were exposed to a combination of TCDD (10 nM) plus \(17\beta\)-estradiol (10 nM) in the presence of various concentrations of 4-hydroxytamoxifen (\(\widetilde{\alpha}\)) or ICI 182,780 (\(\bullet\)) for 48 h. Results are expressed as percentage of activity induced by TCDD (10 nM) plus the antagonist (1000 nM). Enzyme activity in cultures exposed to TCDD plus tamoxifen was 49.78 ± 2.95 pmol/mg of protein/min, and TCDD plus ICI 182,780 was 74.9 ± 1.36 pmol/mg of protein/min. CYP1A1 activity was measured as described in Fig. 2. Each data point represents the mean from three separate cultures performed in triplicate.
receptor level could be a factor in determining the mitigation response by 17\(\beta\)-estradiol, we examined each cell line for estrogen receptor content by radioreceptor assay (27), followed by Scatchard analysis (54). We were unable to detect estrogen receptors in either HuE or Hep-3B cells by this method, but both ECC-1 and MCF-7 cells contained similar quantities of estrogen receptor with comparable apparent \(K_d\) values (MCF-7: \(K_d = 0.74 \pm 0.08\) nM, \(B_{\text{max}} = 418 \pm 54\) fmol/mg; ECC-1: \(K_d = 0.74 \pm 0.07\) nM, \(B_{\text{max}} = 282 \pm 38.5\) fmol/mg).2

17\(\beta\)-Estradiol Treatment Decreased CYP1A1 mRNA Levels—To determine whether reduced monoxygenase activity reflected altered CYP1A1 mRNA expression, we examined steady-state levels of CYP1A1 mRNA over time in ECC-1 cells exposed to TCDD and 17\(\beta\)-estradiol by Northern blot analysis. Total RNA collected from cells exposed to saturating concentrations of TCDD and 17\(\beta\)-estradiol showed a reduction of TCDD-induced CYP1A1 mRNA when compared with cells exposed to TCDD alone (Fig. 5). Cells that were not exposed to TCDD did not display detectable CYP1A1 mRNA. Densitometric analysis of the autoradiograph showed that estrogen reduced CYP1A1 mRNA to 35% of TCDD-induced levels after 24 h, in close agreement with the observed decrease in enzymatic activity (Fig. 1B). To determine whether this effect could be reversed, we exposed cultures of ECC-1 cells to combinations of TCDD, 17\(\beta\)-estradiol, and the anti-estrogens used earlier. The estrogen receptor antagonists ICI 182,780 and 4-hydroxytamoxifen each reversed estrogen action at the level of CYP1A1 mRNA and did not affect CYP1A1 induction by TCDD (Fig. 6).

Estrogen Inhibited Dioxin-mediated Transcription of CYP1A1—To investigate whether estrogen exerts control over CYP1A1 mRNA levels by affecting its transcription, we performed transient transfections of ECC-1 with a reporter plasmid containing a portion of the 5’ regulatory sequence for the human CYP1A1 gene (−1612/+292). This segment contains the promoter and at least three functional AhREs (32, 39, 55). When activated by the Ah receptor complex, the promoter drives transcription of the cDNA sequence encoding firefly luciferase. Exposing transiently transfected cells to TCDD increased luciferase activity 24-fold above that measured in the Me\(\_\)SO control (2.1 \(10^3\) versus 49.6 \(10^3\) relative light units/mg of protein). Exposing transfected cells to both TCDD and 17\(\beta\)-estradiol resulted in a 74% decrease in TCDD-induced luciferase activity (Fig. 7). Addition of estrogen receptor antagonists reversed the estrogen-mediated decrease of TCDD-induced transcriptional activation of the reporter plasmid.

To establish whether the decrease in CYP1A1 mRNA occurred at the transcriptional level and whether transcription varied over time, we performed nuclear run-off experiments using nuclei collected from ECC-1 cultures exposed to TCDD alone, or TCDD plus 17\(\beta\)-estradiol, after 1.5, 4, and 12 h. 17\(\beta\)-Estradiol reduced CYP1A1 transcription at each time point examined (Fig. 8A). Densitometric analysis of autoradiographs from three separate experiments showed that TCDD induced CYP1A1 mRNA maximally by 1.5 h of exposure, which was maintained for at least 12 h (Fig. 8B).

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2 M. S. Ricci and W. A. Toscano, unpublished observations.
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Significant difference (*) was observed only between TCDD plus 17β-estradiol and 17β-estradiol alone to TCDD plus the chemicals indicated was performed by ANOVA. Reporter plasmid overnight and exposed to the chemical(s) indicated in "Experimental Procedures."

Fig. 6. Estrogen receptor antagonists reversed 17β-estradiol reduction of TCDD-induced CYP1A1 mRNA. Confluent cultures of ECC-1 cells were exposed for 16 h to the chemicals indicated. Cells were exposed to the following concentrations: 10 nM TCDD, 10 nM 17β-estradiol, 1000 nM 4-OH-tamoxifen, 1000 nM ICI 182,780. Cells were harvested and Northern analysis was performed as described under "Experimental Procedures."

Fig. 7. Transient transfection of ECC-1 cells showed 17β-estradiol-mediated reduction of Ah receptor transcription is reversible by estrogen receptor antagonists. ECC-1 cells were transfected with a plasmid containing a portion of the 5′-regulatory sequence of the CYP1A1 gene (−1612/+282) that drives transcription of firefly luciferase when activated by TCDD. Cells were transfected with 1 μg reporter plasmid overnight and exposed to the chemical(s) indicated in 0.1% Me2SO (DMSO) (10 nM TCDD, 10 nM 17β-estradiol (E2), 1 μM ICI 182,780, 1 μM 4-OH-tamoxifen (TAM)) for 48 h. Cells were lysed, and relative luciferase activity was measured and normalized to protein content. Each column represents the mean from three separate cultures performed in triplicate ± S.E. Statistical analysis comparing TCDD alone to TCDD plus the chemicals indicated was performed by ANOVA. Significant difference (*) was observed only between TCDD plus 17β-estradiol and TCDD alone (p < 0.004).

Induction of CYP1A1 monoxygenase activity is a hallmark of dioxin alteration of gene expression. In this study, we demonstrated that cultured human endometrial cells exposed to 17β-estradiol reduced dioxin-induced CYP1A1 transcription, mRNA steady-state levels, and enzymatic activity compared with cultures exposed to TCDD alone. The reduction by estrogen was observed in conjunction with the first appearance of CYP1A1 and was persistent. Estrogen action was concentration-dependent and was reversible by estrogen receptor antagonists, which strongly indicates estrogen receptor involvement. Exposure of ECC-1 cultures to other steroid hormones did not show an inhibitory action on TCDD induction of CYP1A1 activity. Estrogen down-modulation of CYP1A1 activity was also cell type-specific. MCF-7 breast cells and ECC-1 endometrial cells, derived from estrogen-sensitive tissues and containing comparable levels of estrogen receptor, both showed decreased CYP1A1 activity in the presence of 17β-estradiol. 17β-Estradiol did not, however, affect CYP1A1 activity in either Hep-3B human liver cells or HuE human keratinocytes, cells that are not normally regulated by estrogen and that had undetectable levels of estrogen receptor.

**DISCUSSION**

The data suggest a linear relationship exists between CYP1A1 transcription, steady-state message levels, and expression of functional enzyme that was uniformly affected by exposure of ECC-1 cultures to 17β-estradiol.

**Gel Mobility Shift Assay**—We performed a gel mobility shift assay to determine whether the observed alteration in CYP1A1 transcription resulted from reduced binding of Ah receptor to AhREs using nuclear extracts from cultures exposed to TCDD alone or to TCDD and 17β-estradiol (Fig. 9). The data indicate a minimal decrease in AhRE binding by nuclear proteins from ECC-1 cultures exposed to TCDD and 17β-estradiol compared with cultures exposed to TCDD alone. Statistical analysis of areas of shifted bands from densitometry indicated no significant difference in DNA binding from nuclear extracts of ECC-1 cells exposed either to TCDD alone or to TCDD plus 17β-estradiol. This observation suggests that the estrogen receptor does not interfere with the ability of the Ah receptor to interact with AhRE.

**17β-Estradiol Does Not Affect CYP1B1 mRNA Levels**—To examine whether the observed decrease in CYP1A1 activity, message, and transcription rate was specific to CYP1A1, or a generalized action, we examined whether 17β-estradiol affected the mRNA level of another TCDD-regulated gene, CYP1B1. We used Northern blot analysis to assess steady-state levels of CYP1B1 message in RNA extracted from ECC-1 cultures exposed to TCDD alone, or TCDD plus various concentrations of 17β-estradiol for 24 h (Fig. 10). The level of CYP1B1 mRNA was not altered by exposure of the cells to 17β-estradiol, but the CYP1A1 message levels decreased as a function of 17β-estradiol concentration, indicating the effect was specific to the CYP1A1 gene. This experiment was performed twice with similar results.

**Recovery of TCDD-mediated Transcription**—The promoter sequences of CYP1A1 and CYP1B1 contain several AhRE sites and binding sites for the SP-1 general transcription factor (56–58). Transcription of CYP1A1 is regulated, in part, by the NF-1 transcription factor, but CYP1B1 is not (56). In addition, NF-1 functions synergistically with estrogen receptor to activate transcription driven by that receptor (59). Because the estrogen receptor and NF-1 interact, we hypothesized that activated estrogen receptor may sequester NF-1 thereby reducing ability of the Ah receptor complex to induce CYP1A1. To test this hypothesis, we examined whether overexpression of NF-1 in ECC-1 cells would overcome the inhibitory effect of 17β-estradiol. ECC-1 cells were co-transfected with the luciferase reporter plasmid containing the 5′ regulatory region of human CYP1A1, described above, and a plasmid containing the cDNA for NF-1 under transcriptional control of the cytomegalovirus promoter (pCMV-NF1). Overexpression of NF-1 reversed 17β-estradiol mediated reduction of TCDD-induced transcription (Fig. 11).

**FIG. 6.** Estrogen receptor antagonists reversed 17β-estradiol reduction of TCDD-induced CYP1A1 mRNA. Confluent cultures of ECC-1 cells were exposed for 16 h to the chemicals indicated. Cells were exposed to the following concentrations: 10 nM TCDD, 10 nM 17β-estradiol, 1000 nM 4-OH-tamoxifen, 1000 nM ICI 182,780. Cells were harvested and Northern analysis was performed as described under “Experimental Procedures.”

**FIG. 7.** Transient transfection of ECC-1 cells showed 17β-estradiol-mediated reduction of Ah receptor transcription is reversible by estrogen receptor antagonists. ECC-1 cells were transfected with a plasmid containing a portion of the 5′-regulatory sequence of the CYP1A1 gene (−1612/+282) that drives transcription of firefly luciferase when activated by TCDD. Cells were transfected with 1 μg reporter plasmid overnight and exposed to the chemicals indicated in 0.1% Me2SO (DMSO) (10 nM TCDD, 10 nM 17β-estradiol (E2), 1 μM ICI 182,780, 1 μM 4-OH-tamoxifen (TAM)) for 48 h. Cells were lysed, and relative luciferase activity was measured and normalized to protein content. Each column represents the mean from three separate cultures performed in triplicate ± S.E. Statistical analysis comparing TCDD alone to TCDD plus the chemicals indicated was performed by ANOVA. Significant difference (*) was observed only between TCDD plus 17β-estradiol and TCDD alone (p < 0.004).
That dioxin decreases many actions mediated by the estrogen receptor is well established (60–62). Reciprocal interaction between estrogen receptor and Ah receptor was reported in MCF-7 human cells and Hepa 1c1c7 murine liver cells (25). The authors suggested that each receptor acted to reduce the ability of the other to bind their respective response elements. A subsequent report, however, disputed whether estrogen affected dioxin action and showed that estrogen does not affect Ah receptor function in the same cell systems (63). Our findings support the observation that estrogen receptor disrupts dioxin-induced CYP1A1, not by altering Ah receptor binding to DNA but by a mechanism involving the general transcription factor NF-1. Using gel mobility shift analysis, we showed that 17β-estradiol did not significantly alter TCDD-activated Ah receptor binding to its DNA-responsive element. To corroborate the functional significance of this observation, we examined......
whether 17β-estradiol altered expression of another dioxin-regulated gene, CYP1B1. Estrogen modulation of dioxin was specific to CYP1A1 but did not affect CYP1B1 mRNA levels, which suggests that Ah receptor binds to AhREs unhindered by 17β-estradiol.

Two regions of the CYP1A1 5′-untranslated region control its transcription. The AhRE-containing dioxin-responsive enhancer begins several hundred base pairs upstream of the transcriptional start site, and the promoter region, containing binding sites for general transcription factors (SP-1 and NF-1), is located immediately upstream of the transcriptional start site (Ref. 64 and references therein). Like CYP1A1, CYP1B1 contains multiple Ah receptor binding sites and binding sites for SP-1 (57). One apparent difference between these two genes is that the promoter for CYP1A1 contains two NF-1 binding sites, of the sequence CCAAT, but CYP1B1 does not (58). In fact, deletion of the NF-1 site proximal to the transcriptional start site of CYP1A1 reduces transcriptional activation by the Ah receptor 80% (56). We deduced that estrogen inhibition of CYP1A1 activity could be mediated by loss of NF-1 function.

When NF-1 was overexpressed in ECC-1 cells, we observed reversal of estrogen action on TCDD-activated CYP1A1 (Fig. 11). We also examined whether overexpression of SP-1 or the co-activating protein p300, both of which are involved in Ah receptor and estrogen receptor mediated transcription (61, 65, 66), could reverse estrogen action, but we did not observe an effect.2

NF-1 is associated with both Ah receptor-mediated and estrogen receptor-mediated transcription. NF-1 synergizes with the estrogen receptor to mediate transcription (59, 67, 68). Our data suggest that when 17β-estradiol activates the estrogen receptor, recruitment of NF-1 by Ah receptor to CYP1A1 is altered, and the factor is directed to 17β-estradiol-responsive genes, resulting in reduction of CYP1A1 induction. Similar to the action of NF-1 with estrogen and other steroid receptors, Ah receptor activates CYP1A1 transcription by modification of chromatin structure in the promoter region, allowing access of NF-1 and other factors to bind and initiate transcription (44, 56, 64, 69). Our observation raises the question of how estrogen receptor appears to sequester available NF-1. It is possible that the nuclear location of ligand-free estrogen receptor versus cytosolic location of ligand-free Ah receptor provides access for the estrogen receptor to direct NF-1 away from dioxin-sensitive CYP1A1 before the Ah receptor complex enters the nucleus or is able to initiate transcription. The relative abundance of estrogen receptor (280 fmol/mg of cytosolic protein) compared with Ah receptor (2 fmol/mg of cytosolic protein) (27) in ECC-1 cells may also contribute to this response.

The observation that CYP1A1 induction was affected by exposing ECC-1 cultures to 17β-estradiol but CYP1B1 was not may be important to the physiology of endometrial cell function. Down-modulation of CYP1A1 by 17β-estradiol could account for the observation that the major endometrial metabolite of 17β-estradiol is the 3,4-catechol derivative, a product of CYP1B1 monooxygenase activity (70, 71). It is possible that Ah receptor is present in the endometrium to act as a mediator of estrogen metabolism. If so, our observation that estrogen can reduce Ah receptor-mediated transcription of CYP1A1 suggests communication exists between the estrogen receptor and the Ah receptor to maintain estrogen homeostasis in estrogen-sensitive tissue. We used TCDD in our study because enzymes induced by the Ah receptor do not metabolize it. Therefore, its effects are persistent and pronounced compared with those induced by other metabolizable ligands for the Ah receptor, which include compounds of dietary origin, such as substituted carbazoles and indole carbinols found in various vegetables, as well as polycyclic aromatic compounds (72–74). The discovery of nontoxic Ah receptor agonists has added to growing evidence that Ah receptor may have other important physiological functions, in addition to mediating xenobiotic metabolism (see Ref. 19 and references therein).

If dietary ligands are important directors of Ah receptor action, then it is possible that estrogen metabolism is governed, in part, by CYP1A1 induced by these ligands. For example, in human subjects exposed to indole-3-carbinol, estradiol 2-hydroxylation is increased 1.5-fold (72). The 2-C derivative of 17β-estradiol hydroxylation is the major estrogen metabolite of CYP1A1 activity (75, 76). This derivative is a less potent estrogen receptor ligand than 17β-estradiol and is a precursor to the major urinary estrogen metabolite, 2-methoxyestrone (16). Therefore, dietary ligands may exert normal homeostatic control over estrogen function by inducing enzymes that regulate estrogen levels. When a toxic, nonmetabolizable ligand, such as TCDD, binds Ah receptor, overproduction of CYP1A1 could occur, leading to observed decreases in estrogen action (77–79). Presence of high levels of estrogen receptor in cells from estrogen-sensitive tissue could mitigate this Ah receptor-induced response, thereby maintaining levels of 17β-estradiol and preserving estrogen homeostasis.

The data presented here show that estrogen exerted significant, immediate, and reversible action on reducing TCDD-induced CYP1A1 transcription and subsequent activity in human endometrial cells. Our data suggest that the ligand-activated estrogen receptor can down-regulate a pathway of estrogen metabolism, and this effect has significant implications for understanding both estrogen receptor-associated disease and Ah receptor-mediated toxicity.

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M. Stacey Ricci, Diane G. Toscano, Carolyn J. Mattingly and William A. Toscano, Jr.

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