THE JOURNAL OF BIOLOGICAL CHEMISTRY
Vol. 274, No. 6, Issue of February 5, pp. 3430–3438, 1999

Printed in U.S.A.

Estrogen Receptor Reduces CYP1A1 Induction in Cultured Human Endometrial Cells*

(Received for publication, November 9, 1998)

M. Stacey Ricci‡, 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

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 17β-estradiol for up to 72 h, with a half-maximal effective concentration (EC_{50}) of 0.9 nM. Reduced enzyme activity was correlated with decreased CYP1A1 mRNA levels, and transcription. Exposure to TCDD plus 17β-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-[9-(4,4,5,5,5-pentafluoro-pentylsulfinyl)nonyl]estra-1,3,5(10)-triene3, 17β-diol restored TCDD-induced *CYP1A1* transcription, steady-state mRNA levels, and enzymatic activity in ECC-1 cells. Gel mobility shift assay showed that 17β-estradiol had little effect on Ah receptor binding to its DNA-responsive element. 17β-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 17β-estradiol antagonism of dioxin induced-CYP1A1. The data suggest that 17β-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,[1] 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 argued 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 17β-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 17β-estradiol hydroxylation in some cells.

Conversely, estrogen appears to mitigate Ah receptor-mediated cytochrome P450 monooxygenase activity in many differ-

* This work was supported in part by the Cancer Association of Greater New Orleans and by funds from the Department of Defense. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

This work id dedicated to Dr. Richard A. Hartline

‡ Recipient of a predoctoral fellowship from the Center for Bioenvironmental Research Fund.

§ To whom correspondence should be addressed: Dept. of Environmental Health Sciences, Tulane School of Public Health & Tropical Medicine SL-29, 1430 Tulane Ave., New Orleans, LA 70112-2699. Tel.: 504-582-7904; Fax: 504-585-6939; E-mail: wtoscan@tmc.tulane.edu.

[1] The abbreviations used are: TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; Ah, aryl hydrocarbon; AhRE, Aryl hydrocarbon response element; CYP1A1, cytochrome P450 1A1; CYP1B1, cytochrome P450 IB1; ECOD, 7-ethoxycoumarin-*O*-deethylase; ICI 182,780, 7α-[9-(4,4,5,5,5-pentafluoro-pentylsulfinyl)nonyl]estra-1,3,5(10)-triene-3,17β-diol; NF-1, nuclear factor 1; SA, specific activity; DTT, dithiothreitol; ANOVA, analysis of variance.

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 *Rsa*I, *Hin*dIII, *Pvu*II, *Sac*I, *Pst*I, *Pvu*II, *Sac*I *Xho*I, *Bsp*EI, *Nhe*I, and Klenow fragment (3′→5′ exo$^-$) and T4 DNA ligase were purchased from New England Biolabs, Beverly, MA. Restriction enzymes *Bgl*II and *Bam*HI and T7 RNA polymerase, DNA polymerase I large Klenow fragment, and pGL3-Basic vector were from Promega (Madison, WI). [α-^{32}P]ATP (SA = 4000 Ci/mmol), [α-^{32}P]UTP (SA = 3000 or 600 Ci/mmol), and [α-^{32}P]dCTP (SA = 3000 Ci/mmol) were purchased from ICN (Costa Mesa, CA). Unless specified otherwise, all other reagents were purchased from commercial sources and used without further purification.

Cells and Culture Conditions—ECC-1 cells were generously provided by Dr. P. G. Satyaswaroop (Milton S. Hershey Medical School, Pennsylvania State University, Hershey, PA), who derived the cell line from a human adenocarcinoma of endometrial epithelium. HuE, a primary keratinocyte cell line, was derived from human neonatal foreskin in this laboratory (28). Hep-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 murine 3T3 fibroblasts, which mimic endometrial stroma and enhance attachment. ECC-1 cells grown in this manner typically reached confluence after 8 days. ECC-1, MCF-7, and HuE cells were all cultivated 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 nM insulin (Sigma). Cells were grown in an atmosphere of 5% CO_2/95% 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 nM 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 Me_2SO unless otherwise stated. Me_2SO 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 the standard. The minimum detectable protein level was 1 ng.

ECOD Assay—The method used to measure cytochrome P450 activity is a modification of published procedures from this laboratory (31). 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 $MgCl_2$, 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% (w/v) trichloroacteic acid. 7-Hydroxycoumarin formed by the catalytic dealkylation reaction was extracted into chloroform (2 ml) by vigorous shaking followed 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 by a Shimadzu spectrofluorometer RF-5301PC (Tokyo, Japan) (λ_{ex} = 368 nm; λ_{em} = 456 nm), and 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.

Radiolabeling of Antisense RNA Probes for Northern Blot Analysis—A plasmid containing the human *CYP1A1* gene (pBS1A1) was the 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 *Rsa*I 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. A plasmid containing the 36B4 cDNA (p36B4) was the generous gift of Dr. Gary Fisher (University of Michigan, Ann Arbor, MI). The 36B4 cDNA codes for human acidic ribosomal phosphoprotein PO and was used as a loading control because it is not regulated by 17-β estradiol (34). A 2.3-kilobase 36B4 DNA fragment containing the T7 RNA polymerase promoter was removed from p36B4 by digesting with *Rsa*I. This fragment produced a 250-base pair riboprobe when transcribed with T7 RNA polymerase. Riboprobes 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 $MgCl_2$; 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-^{32}P]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-^{32}P]UTP by mini-column chromatography using Bio-Gel P60 (Bio-Rad) and used in hybridization procedures.

Radiolabeling of cDNA for Northern Blot Analysis—The pBS1A1 plasmid was digested with *Hin*dIII to obtain a 1.5-kilobase fragment of human CYP1A1 cDNA. A plasmid containing the human *CYP1B1* gene (phCYP1B1-clone 1) was the generous gift of Dr. Thomas Sutter (The Johns Hopkins University) (18). A 1.3-kilobase CYP1B1 cDNA fragment was removed from phCYP1B1 by digestion with *Pvu*II and *Sac*I. The p36B4 plasmid was digested with *Pst*I 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 in the presence of random DNA hexamers (2 μg) by boiling for 3 min and immediately submerging into ice. A mixture containing (final concentration) 0.05 mM dATP/dGTP/dTTP, 50 μCi [α-^{32}P]dCTP (SA = 3000 Ci/mmol), 20 units of Klenow fragment (3′→ 5′ exo$^-$), 10 mM Tris-HCl, pH 7.5, 5 mM $MgCl_2$, and 7.5 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-^{32}P]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/6.4 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 (36)). Membranes were hybridized for 16 h with 10^6 cpm/ml of the riboprobes at 60° C or at 43° C with the DNA probes in prehybridization solution. 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).

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. Fujii-Kuriyama) was digested with *Pvu*II to obtain a 1904 base pair fragment (−1612/+292) and ligated into the *Sma*I site of the BluescriptSK(+) vector (Strategene, La Jolla, CA) using T4 DNA ligase. This portion of the 5′-untranslated region contains the promoter, exon 1, and at least three functional AhREs (32, 39). The AhR-responsive fragment was removed by digestion with *Sac*I and *Xho*I and inserted into the pGL3-Basic vector upstream of the cDNA encoding firefly luciferase. This plasmid, pGL3–5′1A1, 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). Dr. Gordon Hager (National Insititutes of Health, Bethesda, MD) generously supplied a plasmid containing NF-1 (pEGFP-NF1). pEGFP-NF1 was digested with *Bsp*EI and *Nhe*I to remove the cDNA encoding green fluorescent protein from pEGFP-C1 (CLONTECH, Palo Alto, CA). The ends were filled using DNA polymerase I large Klenow fragment and ligated. The plasmid was designated pCMV-NF1. The cDNA encoding NF-1 was removed from pCMV-NF1 by digestion with *Bgl*II and *Bam*HI, ligated, and designated pCMV.

One day prior to transfection, ECC-1 cells were plated on 6-well dishes at a density of 5×10^6/well. Cells were transfected using a cationic lipid-mediated system with 1 μg of pGL3–5í1A1 and 6 μl of LipofectAMINE (Life Technologies, Inc.) overnight. Transfections were performed according to manufacturer's protocol. In experiments using NF-1, cells were transfected with either 1 μg of pCMV-NF1 or an equimolar amount of the empty expression vector, pCMV, plus pBluescriptSK(+) to equalize DNA concentration. After transfection, cells were exposed to the chemical(s) indicated for 48 h. Cells were rinsed twice in phosphate-buffered saline and lysed on the plate by addition of 150 μl of lysis buffer (25 mM Tris-phosphate, pH 7.8, 2 mM DTT, 2 mM EDTA, 10% glycerol, 1% Triton X-100). Cell lysates were collected by scraping into microcentrifuge tubes and centrifuged for 20 s at 12,000 rpm. One hundred microliters of luciferin-containing buffer (0.3 mM beetle luciferin, 0.5 mM acetyl-CoA, 2 mM ATP in 30 mM glycylglycine, pH 7.8, containing 15 mM $MgCl_2$, 0.5 mM DTT) were added to 10 μl of supernatant. Luciferase activity was measured in a luminometer (Monolight 2000, Analytical Luminescence Laboratory, Ann Arbor, MI). Luciferase assays were performed in triplicate, and relative light units were normalized to protein content.

Nuclear Run-off Transcription Assay—Nuclei were collected by sucrose gradient centrifugation, and the run-off assay was performed essentially as described by Marzluff and Huang (38) with some modifications. Five dishes (100 mm) of confluent cells were exposed to the chemical(s) for the time indicated in the legends to Figs. 5, 6, and 10, washed twice with phosphate-buffered saline, collected into a swelling buffer (200 μl of 25 mM KCl, 10 mM HEPES, pH 7.6, 2 mM magnesium acetate, 1 mM EDTA, 3 mM $CaCl_2$, 1 mM DTT, 1 mM phenylmethylsulfonyl fluoride, 10 μM leupeptin) using a rubber policeman, and placed directly into a glass homogenizer. An equal amount of a sucrose-containing buffer was added (0.32 M sucrose, 3 mM $CaCl_2$, 2 mM magnesium acetate, 0.1 mM EDTA, 10 mM Tris, pH 8.0, 1 mM DTT, 1 mM phenylmethylsulfonyl fluoride, and 10 μM leupeptin), and cells were allowed to swell for 5 min. Cells were broken in a Dounce homogenizer, and the cell homogenate was layered onto 20 ml of sucrose cushion buffer (0.88 M sucrose, 2 mM magnesium acetate, 0.1 mM EDTA, 10 mM Tris, 3 mM $CaCl_2$). The sucrose layers were centrifuged at $1500 \times g$ for 15 min. The resulting pellet contained nuclei, which were suspended in an equal volume of a glycerol buffer (40% glycerol, 20 mM Tris, pH 8.0, 10 mM $MgCl_2$ and 1 mM DTT). More than 1×10^7 nuclei were used in each run-off reaction. The reaction mixture consisted of 10 mM Tris, pH 7.5, 0.1 mM EDTA, 80 mM KCl, 5 mM $MgCl_2$, 1 mM DTT, 100 units/ml creatine kinase, 5 mM each of ATP, CTP, and GTP, and 100 μCi of [α-^{32}P]UTP (SA = 3000 Ci/mmol) and was incubated for 30 min at 30° C. Total RNA was isolated using Tri Reagent® RNA isolation reagent (Molecular Research Center, Inc.). RNA was hybridized to 5 μg of a linearized plasmid containing *CYP1A1* cDNA, human β-actin, or the plasmid vector alone, that was immobilized on Zeta Probe® GT nylon membrane (Bio-Rad), according to manufacturer's instructions, using a slot blot apparatus (Schleicher & Schuell). Hybridization, washing, and autoradiography was performed as described for Northern blot analysis, except that hybridization temperature was 42° C and was continued for 72 h. Films were exposed for up to 3 weeks.

Ah Receptor Gel Shift Assay—Dioxin-induced binding of Ah receptor to DNA was performed using a published method (40). The DNA probe was made by annealing two oligonucleotides, 5′-GATCCGGCTCTTCT-CACGCAACTCCGAGCTCA-3′ and 5′-GATCTGAGCTCGGAGT-TGC-GT-GAGAAGAGCCA-3′, previously shown to bind AhR, and end-labeled with [γ-^{32}P]ATP and T4 polynucleotide kinase (41, 42). Cells were exposed to TCDD (10 nM) in the presence or absence of 17β-estradiol (10 nM) in 0.1% Me_2SO for 1 h. Nuclear extracts (60 μg) were incubated with 100,000 cpm of ^{32}P-labeled AhRE oligonucleotide (43) for 15 min in the presence of 6 μg of herring sperm DNA. Samples were analyzed using nondenaturing gel electrophoresis (40). Phosphorimaging was performed using a Fuji FLA-200 phosphorimager for quantitative analysis of shifted bands, and gels were also exposed to Kodak Biomax™ MS double emulsion film for 2 days in order to obtain a permanent copy.

Statistical Analysis—Significant differences between treatment groups were determined by analysis of variance using the ANOVA1 macro sheet from Microsoft Excel® software package (Microsoft Corp.).

RESULTS

Ligand-bound Ah receptor mediates induction of cytochrome P450 monooxygenase(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. 1*A*). The EC_{50} for 17β-estradiol modulation of ECOD activity was 0.9 nM, which correlates well with the apparent K_D 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. 1*B*). 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 cells (27). Glucocorticoids tend to enhance Ah receptor induction of CYP1A1, presumably by glucocorticoid receptor binding at 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 elements located within the CYP1A1 gene (48). These data suggest that the steroid hormone-mediated decrease in Ah receptor action 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 reduction of CYP1A1 induction. Two structurally different "anti-estrogens," 4-hydroxytamoxifen and ICI 182,780, alter 17β-estradiol binding to estrogen receptor and were used in these

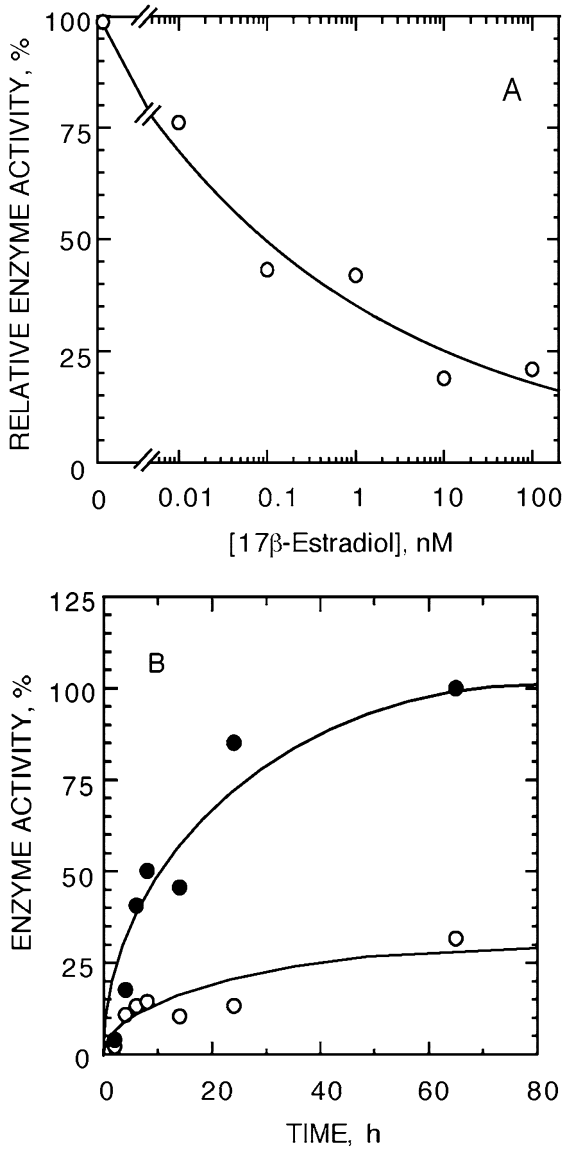

FIG. 1. **17β-Estradiol decreased TCDD-induced cytochrome P450 activity in a concentration-dependent manner.** *A,* confluent cultures of ECC-1 cells were exposed for 48 h to either 10 nM TCDD alone (CYP1A1 specific activity = 109 ± 11.7 pmol/mg of protein/min) or 10 nM TCDD plus 17β-estradiol (○) at 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 (●) or TCDD plus 17β-estradiol (○) (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).

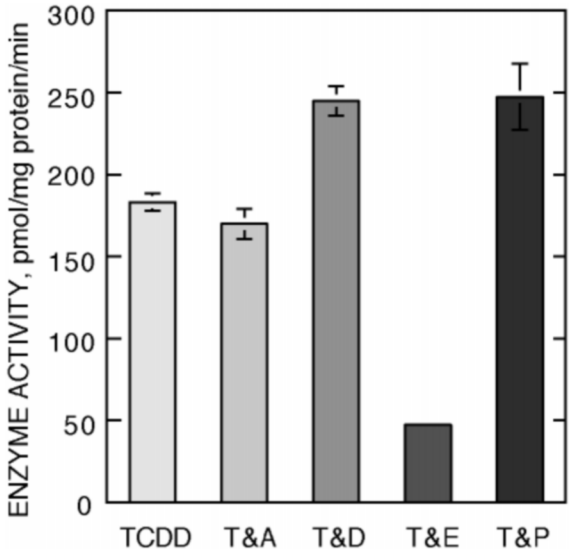

FIG. 2. **Other steroid hormones and steroid receptor agonists do not mediate a decrease in TCDD-induced cytochrome P450 activity.** Confluent cultures were exposed for 48 h to 10 nM TCDD plus 10 nM of the chemical indicated (*A,* 4-androstene-3,17-dione; *D,* dexamethasone; *E,* 17β-estradiol; *P,*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

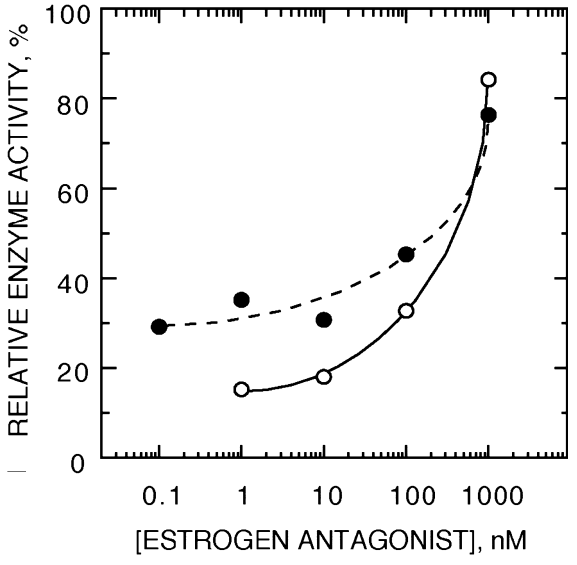

FIG. 3. **Estrogen receptor antagonists reversed 17β-estradiol action and reinstated TCDD induction of cytochrome P450 activity.** Confluent cultures were exposed to a combination of TCDD (10 nM) plus 17β-estradiol (10 nM) in the presence of various concentrations of 4-hydroxytamoxifen (○) or ICI 182,780 (●) 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.

experiments (49, 50). ECC-1 cultures were exposed to combinations of TCDD, 17β-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 17β-estradiol.

Cellular Specificity of 17β-Estradiol Action—To determine whether the observed reduction in CYP1A1 activity was specific to ECC-1 endometrial cells, we examined the effect of 17β-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 17β-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, 17β-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 17β-estradiol (Fig. 4). To confirm that estrogen

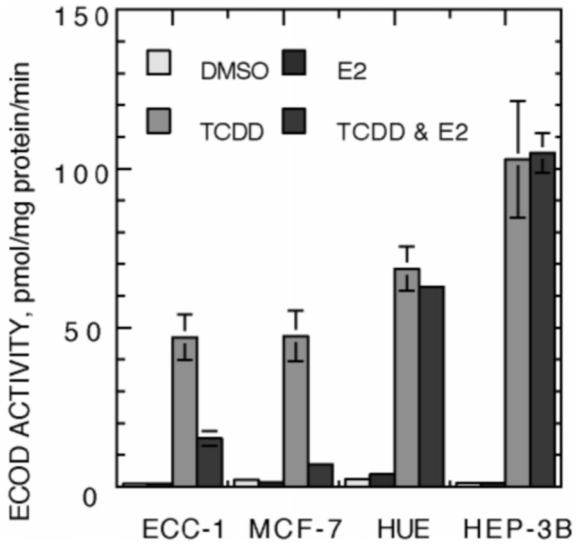

FIG. 4. **17β-Estradiol reduced CYP1A1 activity only in estrogen-regulated cells.** Confluent cultures of ECC-1 human endometrial cells, MCF-7 mammary carcinoma cells, Hep-3B human liver cells, and HuE primary human keratinocyte cells were exposed for 48 h to 10 nM TCDD, with or without 10 nM 17β-estradiol. The solvent control was 0.1% Me_2SO (*DMSO*). CYP1A1 activity was assayed as described in the legend to Fig. 1. The data shown are the mean from three separate cultures performed in triplicate ± S.E.

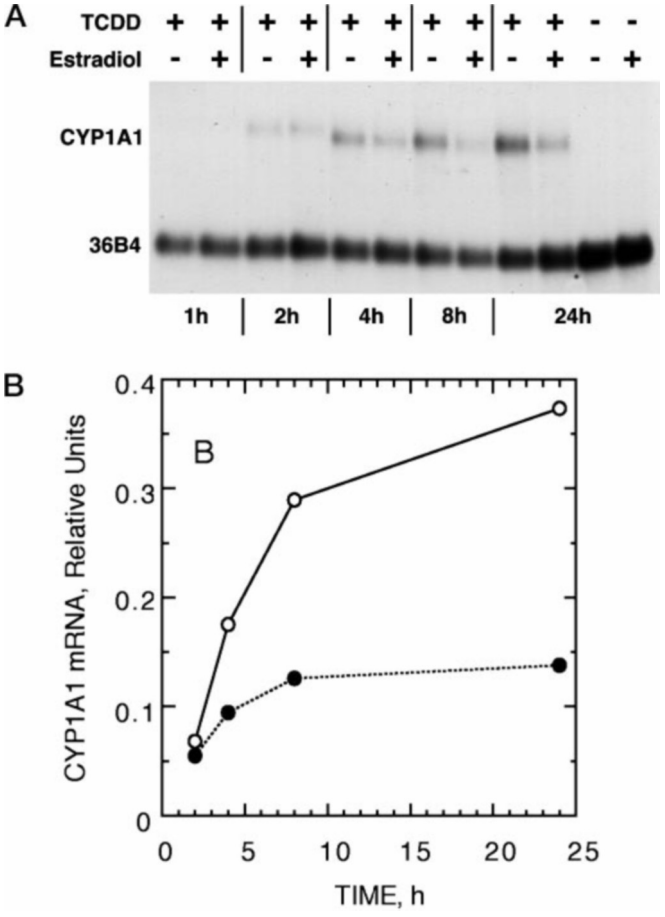

FIG. 5. **17β-Estradiol decreased TCDD-induced CYP1A1 mRNA.** Cells were exposed to TCDD (10 nM) in the presence or absence of 17β-estradiol (10 nM) for the times indicated. Total RNA (5 μg) was separated by electrophoresis on a denaturing agarose gel. The RNA was transferred to a charged nylon membrane and hybridized to an antisense RNA probe for CYP1A1 mRNA. The quantity of RNA loaded was assessed using a probe for the ribosomal protein, 36B4. The time course of mRNA accumulation is in good agreement with induction of enzymatic activity. *A*, autoradiograph of the Northern blot incubated with RNA probes for both CYP1A1 and 36B4 simultaneously. Film was developed after 48 h exposure to blot. *B,* densitometric analysis of Northern blot autoradiograph shown, where the ratio of the quantity of CYP1A1 mRNA (in arbitrary units) induced by TCDD (●) or TCDD plus 17β-estradiol (○), normalized to the quantity of 36B4 mRNA for the sample loaded, is plotted against time of exposure. Ratios for the solvent control (0.1% Me_2SO) and 17β-estradiol (10 nM) were 0.018 and 0.012, respectively.

receptor level could be a factor in determining the mitigation response by 17β-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 ± 0.08 nM, B_{max} = 418 ± 54 fmol/mg; ECC-1: K_d = 0.74 ± 0.07 nM, B_{max} = 282 ± 38.5 fmol/mg).[2]

17β-Estradiol Treatment Decreased CYP1A1 mRNA Levels—To determine whether reduced monooxygenase activity reflected altered CYP1A1 mRNA expression, we examined steady-state levels of CYP1A1 mRNA over time in ECC-1 cells exposed to TCDD and 17β-estradiol by Northern blot analysis. Total RNA collected from cells exposed to saturating concentrations of TCDD and 17β-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. 1*B*). To determine whether this effect could be reversed, we exposed cultures of ECC-1 cells to combinations of TCDD, 17β-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_2SO control (2.1×10^3 *versus* 49.6×10^3 relative light units/mg of protein). Exposing transfected cells to both TCDD and 17β-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 17β-estradiol, after 1.5, 4, and 12 h. 17β-Estradiol reduced CYP1A1 transcription at each time point examined (Fig. 8*A*). 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. 8*B*). Exposure of ECC-1

[2] M. S. Ricci and W. A. Toscano, unpublished observations.

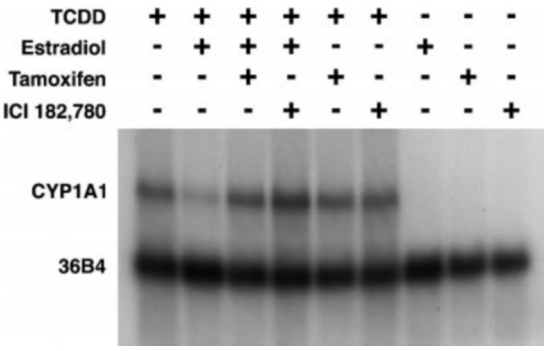

FIG. 6. **Estrogen receptor antagonists reversed 17β-estradiol reduction of TCDD-induced CYP1A1 mRNA.** Confluent cultures of ECC-1 cells were exposed for 16 h to the chemicals indicated. Cells were exposed to the following concentrations: 10 nM TCDD, 10 nM 17β-estradiol, 1000 nM 4-OH-tamoxifen, 1000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

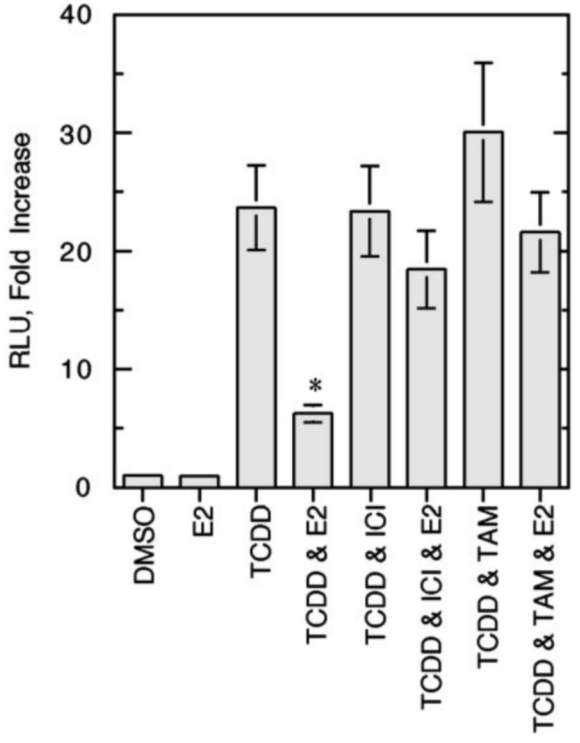

FIG. 7. **Transient transfection of ECC-1 cells showed 17β-estradiol-mediated reduction of Ah receptor transcription is reversible by estrogen receptor antagonists.** ECC-1 cells were transfected with a plasmid containing a portion of the 5′-regulatory sequence of the *CYP1A1* gene (−1612/+292) that drives transcription of firefly luciferase when activated by TCDD. Cells were transfected with 1 μg of reporter plasmid overnight and exposed to the chemical(s) indicated in 0.1% Me_2SO (*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 ($p < 0.004$).

cultures to 17β-estradiol reduced transcription by 60%. 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).

DISCUSSION

Induction of CYP1A1 monooxygenase 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.

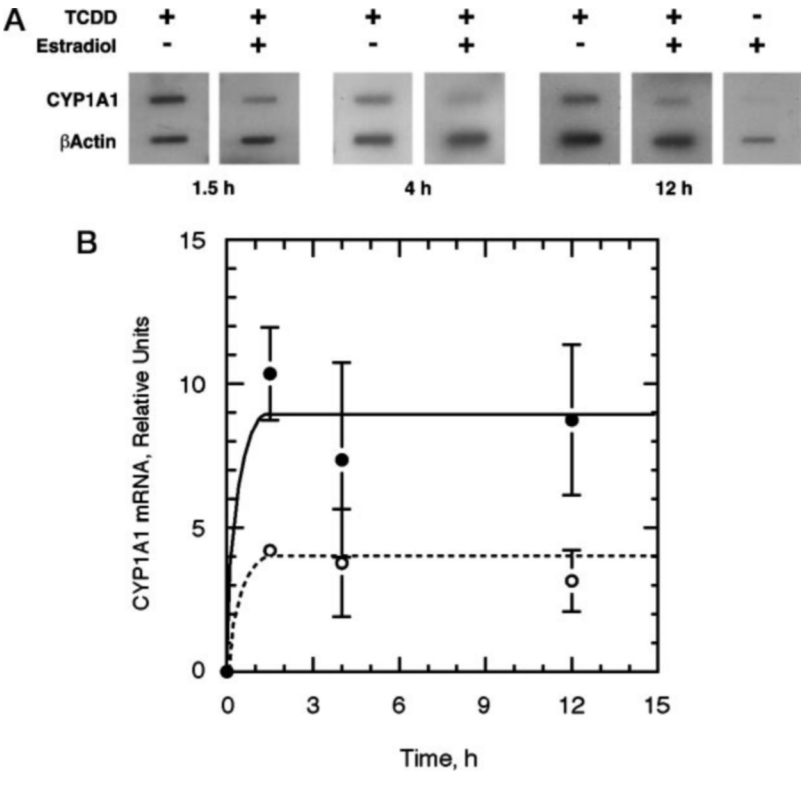

FIG. 8. **Estrogen decreased transcription rate of TCDD-induced CYP1A1.** Confluent cultures were exposed to either TCDD (10 nM) or TCDD plus 17β-estradiol (10 nM) for the times indicated. Nuclei were isolated, and *in vitro* transcription of nascent RNA proceeded in the presence of [α-^{32}P]UTP. Newly transcribed mRNA was isolated and hybridized to CYP1A1 cDNA and human β-actin cDNA. *A,* autoradiograph of membranes after 3 weeks of exposure. *B,* quantitation of densitometric analysis from three separate experiments was carried out as described under "Experimental Procedures." Relative units refer to normalized densitometric volumes (CYP1A1 mRNA/β-actin mRNA) expressed as fold induction above background for TCDD (—●—) and TCDD plus 17β-estradiol (– –○– –).

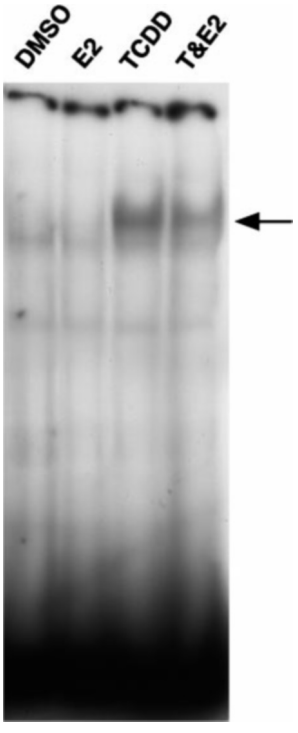

FIG. 9. **17β-Estradiol reduced Ah receptor complex binding to AhRE.** ECC-1 cells were exposed to TCDD (*T*) (10 nM) and 17β-estradiol (*E2*) (10 nM) alone or in combination, or with solvent alone (0.1% Me_2SO (*DMSO*)), for 1 h, after which nuclear protein extracts were prepared. Sixty micrograms of nuclear extract were incubated with ^{32}P-labeled AhRE oligonucleotide probe and subjected to electrophoresis on polyacrylamide gels under nondenaturing conditions. The *arrow* indicates bound AhRE. Statistical analysis of areas obtained from densitometric scans by one-way ANOVA showed no significant differences between the groups exposed to TCDD alone or in the presence of TCDD and 17β-estradiol.

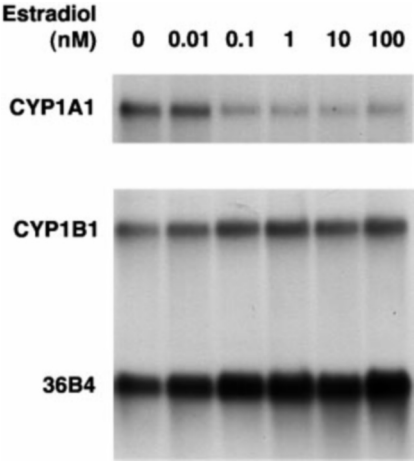

FIG. 10. **17β-Estradiol does not reduce TCDD-induced CYP1B1 mRNA.** Confluent cultures of ECC-1 cells were exposed to 10 nM TCDD plus the indicated concentration of 17β-estradiol for 24 h. Cells were harvested and Northern analysis was performed as described under "Experimental Procedures," except DNA probes rather than riboprobes were used. The blot shown was first incubated with probes for CYP1B1 plus 36B4, placed on film for autoradiography. Later, the blot was stripped and incubated with a CYP1A1 probe and placed on film for autoradiography.

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

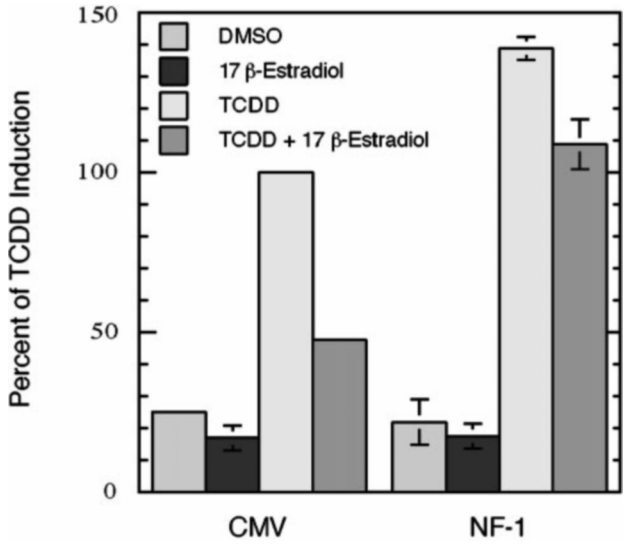

FIG. 11. **Transfection of ECC-1 cells with NF-1 cDNA reverses estrogen receptor-mediated reduction of CYP1A1 transcription.** ECC-1 cells were transfected with 1 μg of NF-1 in a pCMV vector or an equivalent quantity of empty vector as described under "Experimental Procedures." Transfecting medium was replaced with medium containing 17β-estradiol (10 nM); TCDD (10 nM); TCDD (10 nM) plus 17β-estradiol (10 nM); or 0.1% Me_2SO (*DMSO*) as a vehicle control. Cultures were incubated for 48 h, and luciferase activity was assayed as described under "Experimental Procedures." The data shown represent the mean of three separate experiments assayed in triplicate $\pm$ S.E. Differences between groups were significant as analyzed by one-way ANOVA ($p < 0.025$).

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 unhampered 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 was 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.

Acknowledgments—We thank Amy Lavin for expert advice in carrying out gel mobility shift assays. We thank Wayne Backes and John McLachlan for generous use of their fluorometers. We thank Karl Kelsey for his expert assistance in carrying out the NF-1 recovery experiment. We also thank Charles Miller and John McLachlan for critical comments regarding this work.

REFERENCES

1. DeVito, M. J., and Birnbaum, L. S. (1994) in *Dioxins and Health* (Schecter, A., ed) pp. 139–162, Plenum Press, New York
2. Peterson, R. E., Theobold, H. M., and Kimmel, G. L. (1993) *CRC Crit Rev. Toxicol.* **23,** 283–335
3. Schmidt, J. V., and Bradfield, C. A. (1996) *Annu. Rev. Cell Dev. Biol.* **12,** 55–89
4. Reyes, H., Reisz-Porzasz, S., and Hankinson, O. (1992) *Science* **256,** 1193–1195
5. Matsushita, N., Sogawa, K., Ema, M., Yoshida, A., and Fujii-Kuriyama, Y. (1993) *J. Biol. Chem.* **268,** 21002–21006
6. O'Malley, B., and Tsai, M. J. (1993) in *Steroid Hormone Action* (Parker, G. M., ed) pp. 44–63, IRL Press, London
7. Kociba, R. J., Keyes, D. G., Beyer, J. E., Carreon, R. M., Wade, D. A., Dittenberger, D. A., Kalnins, R. P., Frauson, L. E., Park, C. N., Bernard, S. D., Hummel, R. A., and Humiston, C. G. (1978) *Toxicol. Appl. Pharmacol.* **46,** 279–303
8. Bertazzi, P. A., Pesatori, A. C., Consonni, D., Tironi, A., Landi, M. T., and Zocchetti, C. (1993) *Epidemiology* **4,** 398–406
9. Rier, S. E., Martin, D. C., Bowman, R. E., Dmowski, W. P., and Becker, J. L. (1993) *Fundam. Appl. Toxicol.* **21,** 433–441
10. Olive, D. L., and Schwartz, L. B. (1993) *N. Engl. J. Med.* **328,** 1759–1769
11. Bois, F. Y., and Eskenazi, B. (1994) *Environ. Health. Perspect.* **102,** 476–477
12. Koninickx, P. R., Braet, P., Kennedy, S. H., and Barlow, D. H. (1994) *Hum. Reprod.* **9,** 1001–1002
13. Moore, M., Narasimhan, T. R., Wang, X., Krishnan, V., Safe, S., Williams, H. J., and Scott, A. I. (1993) *J. Steroid Biochem. Mol. Biol.* **44,** 251–261
14. Safe, S., Astroff, B., Harris, M., Zacharewski, T., Dickerson, R., Romkes, M., and Biegel, L. (1991) *Pharmacol. Toxicol.* **69,** 400–409
15. Poland, A., Glover, E., and Kende, A. S. (1976) *J. Biol. Chem.* **251,** 4936–4946
16. Martucci, C. P., and Fishman, J. (1993) *Pharmacol. Ther.* **57,** 237–257
17. Quattrochi, L. C., Pendurthi, U. R., Okino, S. T., Potenza, C., and Tukey, R. H. (1986) *Proc. Natl. Acad. Sci. U. S. A.* **83,** 6731–6735
18. Sutter, T. R., Tang, Y. M., Hayes, C. L., Wo, Y.-Y. P., Jabs, E. W., Li, X., Yin, H., Cody, C. W., and Greenlee, W. F. (1994) *J. Biol. Chem.* **269,** 13092–13099
19. Whitlock, J. P. (1994) *Trends Endocrinol. Metab.* **5,** 183–188
20. Nebert, D. W., Bausserman, L. L., and Bates, R. R. (1970) *Int. J. Cancer* **6,** 470–480
21. Gallo, M. A., Hesse, E. J., Macdonald, G. J., and Umbreit, T. H. (1986) *Toxicol. Lett.* **32,** 123–132
22. Moore, M., Narasimhan, T. R., Steinberg, M. A., Wang, X., and Safe, S. (1993) *Arch. Biochem. Biophys.* **305,** 483–488
23. Safe, S., and Krishnan, V. (1995) *Toxicol. Lett.* **82/83,** 731–736
24. Li, J. J., and Li, S. A. (1995) in *Chemical Induction of Cancer: Modulation and Combination Effects* (Arcos, J. C., Argus, M. F., and Woo, Y.-T., eds) pp. 397–451, Birkhauser, Boston
25. Kharat, I., and Saatcioglu, F. (1996) *J. Biol. Chem.* **271,** 10533–10537
26. Satyaswaroop, P. G., Zaino, R. J., and Mortel, R. (1983) *Science* **219,** 58–60
27. Ricci, M. S., Toscano, D. G., and Toscano, W. A., Jr. (1999) *In Vitro Cell Dev. Biol.,* in press
28. Rheinwald, J. G. (1989) in *Cell Growth and Division: A Practical Approach* (Baserga, R., ed) pp. 81–94, IRL Press, New York
29. Boulet, A. P., and Fortier, M. A. (1987) *In Vitro Cell Dev. Biol.* **23,** 93–99
30. Peterson, G. L. (1977) *Anal. Biochem.* **83,** 346–356
31. Hudson, L. G., Shaikh, R., Toscano, W. A., Jr., and Greenlee, W. F. (1983) *Biochem. Biophys. Res. Commun.* **115,** 611–617
32. Quattrochi, L. C., and Tukey, R. H. (1989) *Mol. Pharmacol.* **36,** 66–71
33. Postlind, H., Vu, T. P., Tukey, R. H., and Quattrochi, L. C. (1993) *Toxicol. Appl. Pharmacol.* **118,** 255–262
34. Laborda, J. (1991) *Nucleic Acids Res.* **19,** 3998
35. Feinberg, A. P., and Vogelstein, B. (1983) *Anal. Biochem.* **132,** 6–13
36. Hirsch, V., Riedel, N., Kornfeld, H., Kanki, P. J., Essex, M., and Mullens, J. I. (1986) *Proc. Natl. Acad. Sci. U. S. A.* **83,** 9754–9758
37. Ausubel, F., Brent, R., Kingston, R. E., Moore, D. D., Seidman, J. G., Smith, J. A., and Struch, K. (eds) (1990) *Current Protocols in Molecular Biology,* Greene-Wiley, New York
38. Marzluff, W. F., and Huang, R. C. C. (1984) in *Transcription and Translation: A Practical Approach* (Harris, B. D., and Higgins, S. J., eds), pp. 89–129, IRL Press, London
39. Hines, R. N., Mathis, J. M., and Jacob, C. S. (1988) *Carcinogenesis* **9,** 1599–1605
40. Lavin, A. L., Hahn, D. J., and Gasiewicz, T. A. (1998) *Arch. Biochem. Biophys.* **352,** 9–18
41. Denison, M. S., Fisher, J. M., and Whitlock, J. P., Jr. (1988) *J. Biol. Chem.* **263,** 17221–17224
42. Gasiewicz, T. A., Elferink, C. J., and Henry, E. C. (1991) *Biochemistry* **30,** 2909–2916
43. Sambrook, J., Fritsch, E. F., and Maniatis, T. (1989) *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
44. Denison, M. S., and Whitlock, J. P., Jr. (1995) *J. Biol. Chem.* **270,** 18175–18178
45. Greenlee, W. F., and Poland, A. (1978) *J. Pharmacol. Exp. Ther.* **205,** 596–605
46. Rodrigues, A. D., and Prough, R. A. (1991) in *Methods in Enzymology* (Waterman, M. R., and Johnson, E. F., eds) Vol. 206, pp. 423–432, Academic Press, San Diego
47. Prough, R. A., Linder, M. W., Pinaire, J. A., Xiao, G.-H., and Falkner, K. C. (1996) *FASEB J.* **10,** 1369–1377
48. Truss, M., and Beato, M. (1997) *Endocr. Rev.* **14,** 459–479
49. Pink, J. J., and Jordan, V. C. (1996) *Cancer Res.* **56,** 2321–2330
50. Wakeling, A. E., Dukes, M., and Bowler, J. (1991) *Cancer Res.* **51,** 3867–3873
51. Brooks, S. C., Locke, E. R., and Soule, H. D. (1973) *J. Biol. Chem.* **248,** 6251–6253
52. Horwitz, K. B., Costlow, M. E., and McGuire, W. L. (1975) *Steroids* **26,** 785–795
53. Satyaswaroop, P. G., Sivarajah, A., and Zaino, R. J. (1988) in *Progress in Cancer Research and Therapy* (Bresciani, F., King, R. G. B., and Lippman, M. E., eds) pp. 430–435, Raven Press, New York
54. Limbird, L. E. (1986) *Cell Surface Receptors: A Short Course on Theory and Methods*, Martinus Nijhoff, Boston
55. Kubota, M., Sogawa, K., Kaizu, Y., Sawaya, T., Watanabe, J., Kawajiri, K., Gotoh, O., and Fuji-Kuriyama, Y. (1991) *J. Biochem. (Tokyo)* **110,** 232–236
56. Jones, K. W., and Whitlock, J. P., Jr. (1990) *Mol. Cell. Biol.* **10,** 5098–5105
57. Tang, Y. M., Wo, Y.-Y. P., Stewart, J., Hawkins, A. L., Griffin, C. A., Sutter, T. R., and Greenlee, W. F. (1996) *J. Biol. Chem.* **271,** 28324–28330
58. Wo, Y.-Y. P., Stewart, J., and Greenlee, W. F. (1997) *J. Biol. Chem.* **272,** 26702–26707
59. Martinez, E., Dusserre, Y., Wahli, W., and Mermod, N. (1991) *Mol. Cell. Biol.* **11,** 2937–2945
60. Safe, S. H. (1995) *Pharmacol. Ther.* **67,** 247–281
61. Wang, F., Hoivik, D., Pollenz, R., and Safe, S. (1998) *Nucleic Acids Res.* **26,** 3044–3052
62. Zacharewski, T., and Safe, S. H. (1998) in *Reproductive and Developmental Toxicology* (Korach, K. S., ed) pp. 431–448, Marcel Dekker, New York
63. Hoivik, D., Willett, K., Wilson, C., and Safe, S. (1997) *J. Biol. Chem.* **272,** 30270–30274
64. Whitlock, J. P., Jr., Okino, S. T., Dong, L., Ko, H. P., Clarke-Katzenberg, R., Ma, Q., and Li, H. (1996) *FASEB J.* **10,** 809–818
65. Kobayashi, A., Sogawa, K., and Fujii-Kuriyama, Y. (1996) *J. Biol. Chem.* **271,** 12310–12316
66. deMedieros, S. R. B., Krey, G., Hihi, A. K., and Wahli, W. (1997) *J. Biol. Chem.* **272,** 18250–18260
67. Corthesy, B., Cardinaux, J.-R., Claret, F.-X., and Whali, W. (1989) *Mol. Cell. Biol.* **9,** 5548–5562
68. Corthesy, B., Claret, F.-X., and Whali, W. (1990) *Proc. Natl. Acad. Sci. U. S. A.* **87,** 7878–7882
69. Cardinaux, J.-R., Chapel, S., and Wahli, W. (1994) *J. Biol. Chem.* **269,** 32947–32956
70. Liehr, J. G., Ricci, M. J., Jefcoate, C. R., Hannigan, E. V., Hokanson, J. A., and Zhu, B. T. (1995) *Proc. Natl. Acad. Sci. U. S. A.* **92,** 9220–9224
71. Liehr, J. G., and Ricci, M. J. (1996) *Proc. Natl. Acad. Sci. U. S. A.* **93,** 3294–3296
72. Michnovicz, J. J., and Bradlow, H. L. (1990) *J. Natl. Cancer Inst.* **82,** 947–949
73. Kleman, M. I., Poellinger, L., and Gustafsson, J.-A. (1994) *J. Biol. Chem.* **269,** 5137–5144
74. Miller, C. A., III (1997) *J. Biol. Chem.* **272,** 32824–32829
75. Spink, D. C., Lincoln, D. W. I., Dickerman, H. W., and Gierthy, J. F. (1990) *Proc. Natl. Acad. Sci. U. S. A.* **87,** 6917–6921
76. Spink, D. C., Eugster, H. P., Lincoln, D. W., II, Schuetz, J. D., Schuetz, E. G., Johnson, J. A., Kaminsky, L. S., and Gierthy, J. F. (1992) *Arch. Biochem. Biophys.* **293,** 342–348
77. Astroff, B., and Safe, S. (1988) *Toxicol. Appl. Pharmacol.* **95,** 435–443
78. Harris, M., Zarcarewski, T., and Safe, S. (1990) *Cancer Res.* **50,** 3579–3584
79. Krishnan, V., Porter, W., Santostefano, M., Wang, X., and Safe, S. (1995) *Mol. Cell. Biol.* **15,** 6710–6719