Journal of Cellular Biochemistry 70:289–296 (1998)

FAST TRACK

12-*O*-Tetradecanoylphorbol-13-Acetate Upregulates the Ah Receptor and Differentially Alters CYP1B1 and CYP1A1 Expression in MCF-7 Breast Cancer Cells

Barbara C. Spink, Michael J. Fasco, John F. Gierthy, and David C. Spink*

Wadsworth Center, New York State Department of Health, Albany, New York 12201–0509

Abstract Elevated expression of cytochrome P450 1B1 (CYP1B1) and estradiol 4-hydroxylation have been reported to be biomarkers of tumorigenesis in humans. The aromatic hydrocarbon receptor (AhR) regulates expression of human cytochrome P450 1A1 (CYP1A1) and CYP1B1, 17β-estradiol (E_2) 2- and 4-hydroxylases, respectively. There is also evidence that expression of estrogen receptor α (ERα) potentiates CYP1A1 inducibility in breast cancer cells. To characterize these relationships further, we examined the effects of 12-*O*-tetradecanoylphorbol-13-acetate (TPA), which downregulates ERα, and the high-affinity AhR ligand, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), on the expression of AhR, ERα, CYP1A1, and CYP1B1 in MCF-7 human breast cancer cells. Treatment with TPA, which suppressed ERα mRNA levels, caused a greater than fourfold elevation of AhR mRNA and protein levels, whereas treatment with TCDD caused a decrease in AhR protein but no change in ERα or AhR mRNA levels. In MCF-7 cells treated with TPA prior to treatment with TCDD, the AhR mRNA level was elevated, the ERα mRNA level remained suppressed, and the ratio of CYP1B1 to CYP1A1 mRNA was increased compared with treatment with TCDD alone. A corresponding increase in the ratio of the rates of 4- to 2-hydroxylation pathways of E_2 metabolism was also observed in response to pretreatment with TPA prior to the addition of TCDD. These results demonstrate differential regulation of the human *CYP1A1* and *CYP1B1* genes and provide a cellular model to investigate further the mechanisms that may be involved in the elevated expression of CYP1B1 in tumorigenesis. J. Cell. Biochem. 70:289–296, 1998.

Key words: cytochrome P450; estrogen metabolism; estradiol 4-hydroxylation; estrogen receptor; 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; polymerase chain reaction; cancer biomarkers

Roles of cytochromes P450 (P450 or CYP) in both the initiation and promotion phases of carcinogenesis have been proposed. P450s catalyze the metabolic activation of environmental, dietary, and possibly endogenous compounds to molecular forms that through subsequent reactions either directly or indirectly lead to DNA damage and mutations. P450s also catalyze the metabolism of a number of endocrine and autocrine factors including steroids, sterols, icosanoids, and retinoids that regulate the growth and differentiation of both normal and neoplastic cells [Nebert, 1991]. Enhanced P450-catalyzed metabolism of these compounds may inhibit tumor promotion by depressing their intracellular levels and thus reducing their effects on tumor cell proliferation. In studies with human breast-derived cells in vitro, agonists of the aromatic hydrocarbon receptor (AhR), a ligand-activated, basic helix–loop–helix transcription factor, induced P450-catalyzed metabolism of 17β-estradiol (E_2), leading to depression of cellular E_2 levels and reduced estrogenic responses [Spink et al., 1990]. This occurs primarily by enhancing the rate of the innocuous 2-hydroxylation pathway; however, E_2 4-hydroxylation, a pathway associated with carcinogenesis in experimental animals [Liehr et al., 1986; Li and Li, 1987; Weisz et al., 1992] and possibly in humans [Liehr et al., 1995; Liehr and Ricci, 1996], is also elevated by activation of the AhR in some tumor- and nontumor-derived human breast cells [Spink et al., 1998].

Studies with MCF-7 breast cancer cells have shown that the E_2 2- and 4-hydroxylases in-

Contract grant sponsor: National Institutes of Health; Contract grant numbers: CA70553 and ES03561; Contract grant sponsor: Superfund Basic Research; Contract grant number: ES03819.

*Correspondence to: Dr. David C. Spink, Wadsworth Center, New York State Department of Health, P. O. Box 509, Albany, New York 12201–0509.
E-mail: david.spink@wadsworth.org

Received 14 April 1998; Accepted 14 April 1998

duced by the high-affinity AhR ligand, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), were distinct enzymes [Spink et al., 1992]. CYP1A1 [Spink et al., 1992] and CYP1B1 [Spink et al., 1994a; Hayes et al., 1996] were identified as the major AhR-regulated E_2 2- and 4-hydroxylases, respectively. Although both human CYP1A1 and CYP1B1 are under the regulatory control of the AhR, there is evidence for differential regulation of the genes encoding these two enzymes. The CYP1A1 and CYP1B1 mRNAs do not show the same tissue distribution [Shimada et al., 1996], and they are not always expressed in concert in cultured cells [Spink et al., 1994a, 1997, 1998; Christou et al., 1994; Döhr et al., 1995; Kress and Greenlee, 1997]. Elevated expression of CYP1B1 [Murray et al., 1997] and putative CYP1B1-catalyzed E_2 4-hydroxylation [Liehr et al., 1995; Liehr and Ricci, 1996] have been reported to be probable biomarkers of carcinogenesis in several tissues including breast and uterine myometrium. Although in vitro studies with human breast cancer cells have demonstrated differential expression of CYP1A1 and CYP1B1, to date there has been little progress in determining the molecular mechanisms and gene regulatory processes involved.

The level of expression of the AhR is a potential control point for the modulation of expression of CYP1A1 and CYP1B1. From studies of AhR levels evaluated by high-affinity TCDD binding, there is evidence that the level of AhR can be the rate-limiting factor in the induction of CYP1A1, particularly in cells expressing a low constitutive level of AhR [Marie et al., 1988; Swanson and Perdew, 1993]. There is also evidence from transient transfection experiments that CYP1A1 inducibility in some human breast cancer cells may be potentiated by the expression of estrogen receptor α (ERα) [Thomsen et al., 1994]. If levels of expression of the AhR and ERα are important in the regulation of CYP1A1 and CYP1B1, pharmacologic manipulation of the AhR and ERα levels may be reflected in altered expression of CYP1A1 and CYP1B1. Studies with several cell-culture systems in vitro have shown that exposure to 12-*O*-tetradecanoylphorbol-13-acetate (TPA) causes an upregulation of AhR mRNA expression [Hayashi et al., 1995]. TPA is also known to downregulate ERα in MCF-7 cells [Ree et al., 1991; Gierthy et al., 1996]. In studies with the ultimate aim of elucidating the mechanisms of the differential regulation of the *CYP1A1* and *CYP1B1* genes, we investigated the effects of TCDD in combination with TPA on AhR, ERα, CYP1A1, and CYP1B1 expression in MCF-7 human breast cancer cells.

MATERIALS AND METHODS

Cell Culture

MCF-7 cells were maintained at 37°C in a humidified atmosphere containing 5% CO_2 with Dulbecco's Modified Eagle's Medium, without phenol red, supplemented with 5% bovine calf serum and other components as described elsewhere [Gierthy et al., 1987] in T-75 flasks (75 cm^2, 10 ml medium) or six-well plates (10 cm^2 wells, 2 ml medium per well). Treatments were performed in triplicate on confluent cultures by the addition of media containing 0.1% (v/v) dimethyl sulfoxide (DMSO) as a solvent control, 10 nM TCDD (Cambridge Isotope Laboratories, Woburn, MA), or 100 nM TPA (Sigma, St. Louis, MO).

RNA Isolation and Reverse Transcription–Polymerase Chain Reaction (RT-PCR)

Total RNA was isolated [Chomcynski and Sacchi, 1987], and 2.5- to 5-µg portions were primed with oligo-dT, reverse transcribed by using Superscript II reverse transcriptase (Life Technologies, Gaithersburg, MD), and treated with RNAse H (Life Technologies) according to the manufacturer's protocol. Competitor sequences were synthesized by the method of Förster [1994]. The primers for amplifying the cDNA sequences of GAPDH and CYP1A1 and the linker primers for synthesizing the corresponding competitors were as described by Fasco et al. [1995]; the primers and linker primers for AhR and ERα were as described by Spink et al. [1998]. For determination of CYP1B1 mRNA levels by RT-PCR, the forward primer was 5′-GCCACTATCACTGACATCT-3′, nucleotides 1310–1328 [Sutter et al., 1994], the reverse primer was 5′-CTTGCCTCTTGCTTCT-TATT-3′, nucleotides 1974–1993, and the linker primer was 5′-GCTTCTTATTCATTTTCGCAG-GCTCATTTG-3′. Each amplified sequence spanned an exon–exon junction, and all amplified sequences were confirmed by restriction endonuclease digestion.

PCR was carried out with core reagents from Perkin Elmer (Foster City, CA), but with the

addition of *Taq*-start antibody (Clontech, Palo Alto, CA) and *Taq* extender (Stratagene, La Jolla, CA). After removing a template-free control aliquot, competitor was added to the final PCR mix, which was then combined with different amounts of cDNA. These samples were subjected to 24–31 cycles of PCR in a Perkin Elmer 9600 thermal cycler. Each cycle consisted of denaturation at 95°C for 10 s, annealing at 65°C (for amplification of GAPDH cDNA) or 60°C (for amplification of ERα, AhR, CYP1A1, and CYP1B1 cDNAs) for 15 s, and amplification at 72°C for 30 s followed by a final elongation at 72°C for 5 min. Aliquots were slowly cooled to 4°C and separated on a 2% 3:1 Nusieve agarose (FMC Bioproducts, Rockland, ME) gel, followed by staining with 0.75 μg/ml ethidium bromide. Photographic negatives of these gels were scanned by using an LKB Imagemaster densitometer (Pharmacia, Uppsala, Sweden) and, for quantitation, band absorbances were compared with those of mass-ladder standards (Life Technologies). A plot of the log of the amount of input cDNA versus the log of the ratio of the amount of the amplified cDNA product to that of the amplified competitor was used as a calibration curve to determine mRNA levels in unknown treatment samples, as described by Li et al. [1994]. All RNA levels were analyzed in triplicate, each representing three separate reverse transcriptions of RNA isolated from individual flasks of control or treated MCF-7 cells and were amplified with the same PCR-plus-competitor mix used for the calibration curve at dilutions that fell within the linear range. To control for variations in RNA preparations and reverse-transcriptional efficiencies, each determination of mRNA concentration was normalized to the concentration of GAPDH mRNA determined from the same reverse transcription.

Determination of AhR Protein Levels

Total protein from six-well plates of control and TCDD- or TPA-treated MCF-7 cells was prepared and analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and Western immunoblotting as described by Pollenz [1996]. Blots were blocked with 3% bovine serum albumin in phosphate buffered saline containing 0.05% Tween-20 (Sigma) for AhR detection or 0.15% Tween-20 for actin detection. Blots were probed with mouse anti-actin monoclonal antibody (Boehringer Mannheim, Indianapolis, IN) at a 1:2,000 dilution or mouse polyclonal anti-AhR antibody (Affinity Bioreagents, Neshanic Station, NJ) at a 1:500 dilution followed by goat anti-mouse horseradish peroxidase-conjugated antibody (Organon Teknika Corp., Durham, NC) at a 1:10,000 dilution and detected by using the SuperSignal™ enhanced chemiluminescence system (Pierce, Rockford, IL). The AhR and actin signals were quantified by scanning densitometry.

Assay of E_2 Metabolism

Assays of E_2 metabolism with cultures of intact cells were performed essentially as described by Spink et al. [1990, 1994a,b]. Briefly, after cultures were exposed to 10 nM TCDD, 100 nM TPA, or the two agents in combination as indicated, media were replaced with medium containing 1 μM E_2 for 6 h. These media were recovered, and 2-ml aliquots were treated with β-glucuronidase/sulfatase (type H-2, Sigma) for hydrolysis of the metabolite conjugates. These enzyme-treated samples were subjected to solid-phase extraction, and trimethysilyl derivatives of the metabolites were prepared. The metabolite derivatives were analyzed by gas chromatography/mass spectrometry with stable-isotope dilution and selected-ion monitoring.

Statistical Analyses

Results for RNA levels determined by RT-PCR and rates of metabolite formation are given as the means ± standard errors (± SEM). Statistical analyses were performed by using analysis of variance and the Newman-Keuls test for multiple comparisons; significance was determined at the level of $P < 0.05$.

RESULTS

The effects of exposure to 100 nM TPA and 10 nM TCDD on AhR mRNA levels are shown in Figure 1A. Treatment with TPA for 4, 15, and 24 h resulted in four- to sevenfold increases in AhR mRNA levels, whereas treatment with TCDD resulted in no significant change in AhR mRNA levels. To determine whether AhR protein levels were affected by treatment with TPA or TCDD, samples of total cellular protein were analyzed by polyacrylamide gel electrophoresis and immunoblotting. Figure 1B shows a Western immunoblot detection of AhR and actin proteins from cells exposed for 15 h to 100 nM TPA and 10 nM TCDD. The AhR protein mi-

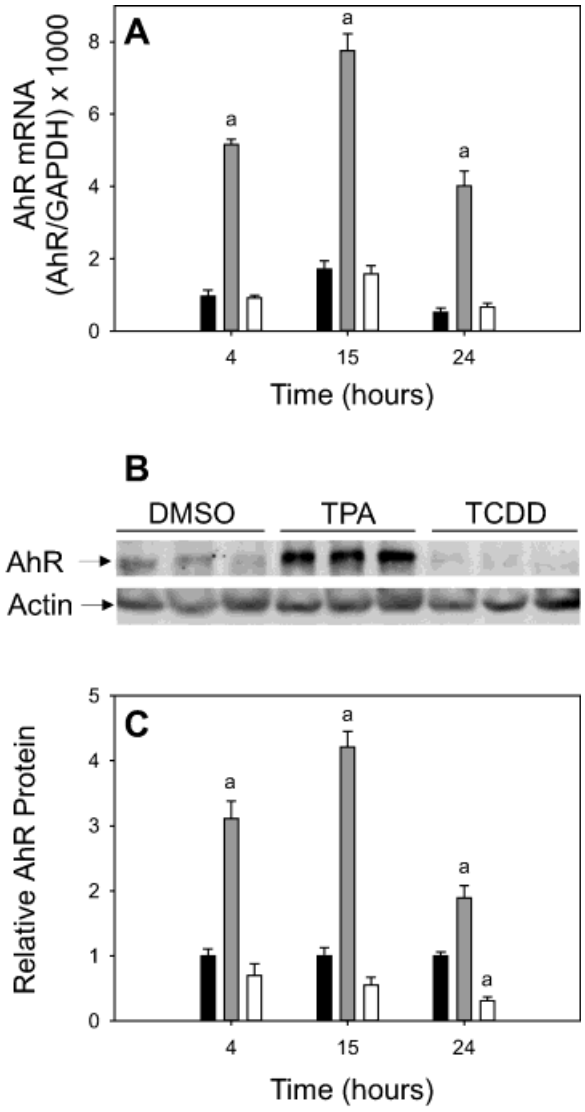

Fig. 1. Effects of exposure to TCDD and TPA on AhR expression in MCF-7 cells. **A:** AhR mRNA levels, expressed relative to GAPDH mRNA levels, were determined by competitive RT-PCR for MCF-7 cultures exposed to the DMSO (0.1% v/v) solvent vehicle (black bars), 100 nM TPA (gray bars), or 10 nM TCDD (open bars) for 4, 15, or 24 h. **B:** Western immunoblots with enhanced chemiluminescence detection of AhR and actin in protein samples from MCF-7 cells exposed to the DMSO (0.1% v/v) solvent vehicle, 10 nM TCDD, and 100 nM TPA for 15 h are shown. **C:** AhR protein levels, expressed relative to actin, are shown for MCF-7 cultures exposed to the DMSO (0.1% v/v) solvent vehicle (black bars), 100 nM TPA (gray bars), or 10 nM TCDD (open bars) for 4, 15, or 24 h. Values are the means ± SEM of three to six determinations. [a]Significantly different from DMSO control ($P < 0.05$).

grated with an apparent molecular mass of 107 kDa, in close agreement with that of human AhR in HeLa cells [Poland and Glover, 1987]. The quantitation of AhR protein levels relative to actin from 4-, 15-, and 24-h exposures to 100 nM TPA and 10 nM TCDD is shown in Figure 1C. Whereas AhR protein levels, similar to the AhR mRNA levels, were elevated when MCF-7 cells were treated with TPA, treatment of cells with TCDD resulted in a decrease of AhR protein levels to about 30% of control levels, despite the fact that AhR mRNA levels remained constant.

Because exposure to TPA was found to alter AhR expression in MCF-7 cells, we determined the effect of TPA on the induction of the AhR-responsive *CYP1A1* and *CYP1B1* genes by TCDD. MCF-7 cell cultures were pretreated for 3 h with or without 100 nM TPA; 10 nM TCDD was then added and the cultures were incubated for an additional 12 h. The levels of CYP1B1, CYP1A1, AhR, ERα, and GAPDH mRNA were subsequently determined by competitive PCR analysis. A typical competitive PCR experiment is shown in Figure 2, in which the CYP1A1 and CYP1B1 cDNAs and their competitors were coamplified and analyzed on a single agarose gel. As indicated in Figure 3A, AhR mRNA was elevated by exposure to 100 nM TPA as before, about fourfold as compared with the DMSO control, whereas treatment with 10 nM TCDD did not affect the AhR mRNA level in the presence or absence of TPA. The level of ERα mRNA was depressed by treat-

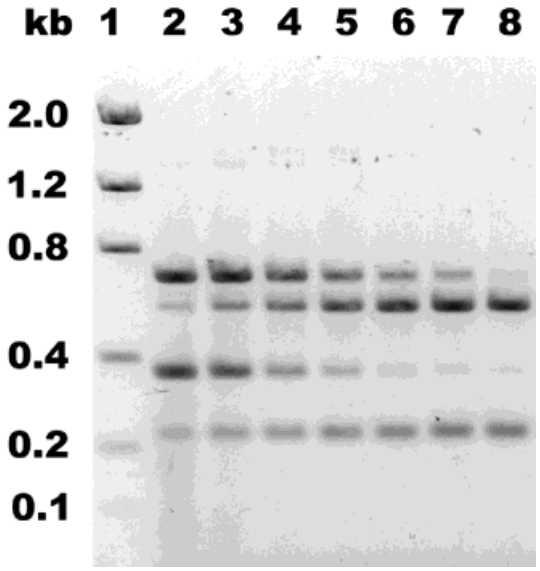

Fig. 2. Analysis of CYP1A1 and CYP1B1 mRNA levels in MCF-7 cells by competitive RT-PCR. Shown are the coamplifications of the 368-bp CYP1A1 sequence with the 219-bp CYP1A1 competitor sequence and the 684-bp CYP1B1 sequence with the 556-bp CYP1B1 competitor sequence. DNA mass-ladder markers are in **lane 1**; **lanes 2–8** represent twofold decreases of input cDNA over the previous lane.

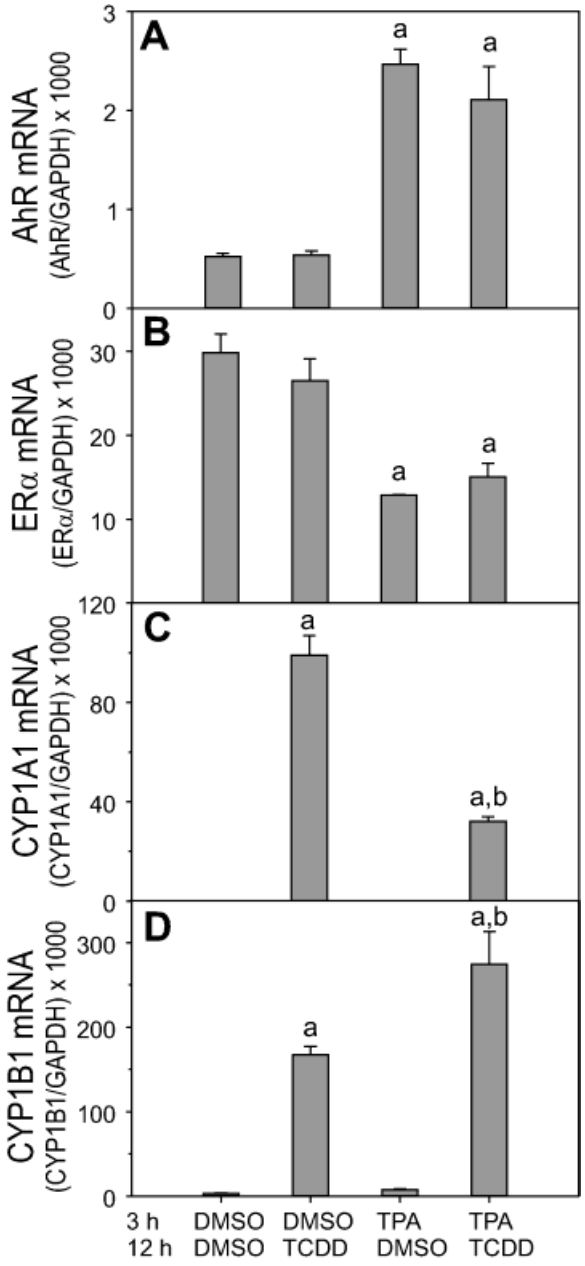

Fig. 3. Effects of combined exposure to TPA and TCDD on the levels of ERα, AhR, CYP1A1, and CYP1B1 mRNA levels. Cultures of MCF-7 cells were exposed to the solvent vehicle, 0.1% v/v DMSO, or 100 nM TPA for 3 h, followed by the addition of DMSO or TCDD, to a final concentration of 10 nM and incubation for an additional 12 h. Total RNA was then isolated and analyzed by competitive RT-PCR for (**A**) AhR, (**B**) ERα, (**C**) CYP1A1, and (**D**) CYP1B1 mRNA and expressed relative to GAPDH mRNA, as described in Materials and Methods. Values are the means ± SEM of three determinations. [a]Significantly different from DMSO/DMSO control; [b]significantly different from DMSO/10 nM TCDD ($P < 0.05$).

ment with 100 nM TPA (Fig. 3B), as has been shown previously [Ree et al., 1991; Gierthy et al 1996]; addition of 10 nM TCDD did not significantly affect the ERα mRNA level in the presence or absence of TPA.

Prior addition of TPA to the medium had differential effects on the TCDD-induced expression of CYP1A1 and CYP1B1 mRNAs. Exposure to 100 nM TPA followed by addition of 10 nM TCDD caused a significant decrease in expression of CYP1A1 mRNA to 32% of that observed with TCDD alone (Fig. 3C). In contrast to the effect on CYP1A1 mRNA, exposure to 100 nM TPA followed by 10 nM TCDD caused a significant increase in expression of CYP1B1 mRNA to 164% of the level of TCDD alone (Fig. 3D). A similar pattern of CYP1A1 and CYP1B1 expression in response to preexposure to TPA followed by addition of TCDD was indicated by assay of E_2 metabolism with intact cells. After a 3-h pretreatment of MCF-7 cells with 100 nM TPA followed by 12-h exposure to 1 or 10 nM TCDD, the rate of metabolism of E_2 was determined. Figure 4 shows that the TCDD-induced 4-methoxyestradiol ($MeOE_2$) formation, a reflection of CYP1B1 activity [Spink et al., 1994a, 1997; Hayes et al., 1996], was significantly ele-

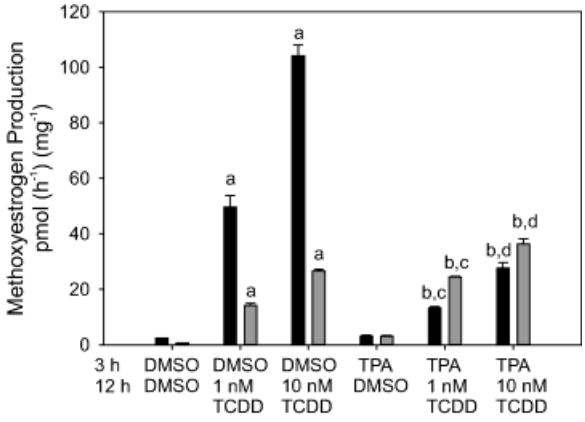

Fig. 4. Effects of combined exposure to TPA and TCDD on 2- and 4-methoxyestradiol formation in MCF-7 cells. Cultures of MCF-7 cells were exposed to the solvent vehicle, 0.1% v/v DMSO, or 100 nM TPA for 3 h, followed by the addition of DMSO or TCDD, to a final concentration of 1 or 10 nM and incubation for an additional 12 h. Media were then replaced by media containing 1 μM E_2. After an additional 6 h, these media were recovered, and estrogen metabolites were analyzed. Rates of 2-$MeOE_2$ (black bars) and 4-$MeOE_2$ (gray bars) production are shown; values are means ± SEM of three determinations. [a]Significantly different from DMSO/DMSO control; [b]significantly different from TPA/DMSO; [c]significantly different from DMSO/1 nM TCDD; [d]significantly different from DMSO/10 nM TCDD ($P < 0.05$).

vated by prior exposure of the cells to TPA. Conversely, the rate of TCDD-induced 2-$MeOE_2$ formation, a reflection of CYP1A1 activity [Spink et al., 1992, 1994a], was significantly decreased relative to the cultures receiving TCDD alone. The depression of the TCDD-induced 2-$MeOE_2$ formation and the elevation of the TCDD-induced 4-$MeOE_2$ formation by TPA were observed at 1 nM TCDD, the approximate EC_{50} for the induction of both 2- and 4-$MeOE_2$ formation [Spink et al., 1994b], and at 10 nM TCDD, a concentration giving maximal induction of formation of both metabolites.

DISCUSSION

In analogy with observations with mouse palatal tissue in vivo [Abbott et al., 1994] and several rodent-derived cell lines in vitro [Pollenz, 1996], a decrease in AhR protein levels was observed when MCF-7 cells were treated with TCDD. This decrease in AhR protein levels in MCF-7 cells was not accompanied by a decrease in AhR mRNA levels, indicating that this TCDD-mediated downregulation of AhR was not at the level of transcription. This downregulation of the AhR may represent a mechanism whereby cells become refractory to continuous stimulation by AhR agonists [Pollenz, 1996]. In contrast to the effect of TCDD, TPA caused a marked elevation of AhR expression in MCF-7 cells at both the mRNA and protein levels. Hayashi et al. [1995] reported a marked increase in the level of AhR mRNA in human HL60 leukemia cells after exposure to TPA; our results indicate a very similar, rapid increase in AhR mRNA and protein levels after exposure to TPA.

In some cases, exposure to TPA leads to elevated rates of AhR-regulated transcriptional activity [Moore et al., 1993; Chen and Tukey, 1996]; however, in other experimental systems, exposure to TPA results in decreases in rates of AhR-regulated gene transcription, in particular that of *CYP1A1* [Raunio and Pelkonen, 1983; Carrier et al., 1992; Okino et al., 1992]. In TPA-treated MCF-7 cells, we observed both enhanced and depressed rates of AhR-regulated gene expression; TCDD-induced expression of CYP1B1 mRNA was augmented, whereas TCDD-induced expression of CYP1A1 was inhibited by prior exposure to TPA. These results indicate distinct molecular mechanisms regulating the expression of the *CYP1A1* and *CYP1B1* genes.

TPA is known to cause a wide spectrum of cellular effects, some of which can be directly related to the increase in the activity of protein kinase C, whereas others appear to be related to AP-1 transcription factor activity. Although there are a number of possible mechanisms, this differential effect of TPA on *CYP1A1* and *CYP1B1* gene expression in human breast cancer cells could be linked to ERα expression. Downregulation of ERα expression in MCF-7 cells by TPA [Ree et al., 1991; Gierthy et al., 1996] and the concomitant cellular changes could influence TCDD-induced *CYP1A1*, but not *CYP1B1*, gene expression by the converse of the mechanism by which elevated ERα expression by transient transfection allows *CYP1A1* promoter activity [Thomsen et al., 1994]. *CYP1B1* gene expression, which appears to be independent of ERα expression [Döhr et al., 1995], was elevated by exposure to TPA, possibly in response to higher AhR levels.

The relative expression of CYP1A1 and CYP1B1 may also reflect phenotypic changes that occur in response to exposure to a tumor promoter such as TPA. Exposure to TPA elicits some of the same changes in MCF-7 cells in vitro that are thought to occur during the development of mammary carcinogenesis, including decreased expression of ERα, changes in cell morphology, and increased cell motility and invasiveness [Platet et al., 1998]. These cellular and molecular changes are concomitant with elevated CYP1B1 and depressed CYP1A1 inducibility by AhR agonists.

In several recent studies, evidence has been presented that elevated expression of CYP1B1 [McKay et al., 1995; Murray et al., 1997] and E_2 4-hydroxylation [Liehr et al., 1995; Liehr and Ricci, 1996] are biomarkers of carcinogenesis in several human tissues including breast. Increased formation of 4-hydroxyestradiol may also have a role in the initiation of carcinogenesis through its conversion to highly reactive seimiquinone and quinone forms, leading to DNA damage and mutations [Liehr and Roy, 1990; Yager and Liehr, 1996; Cavalieri et al., 1997]. This is the first report indicating that expression of the *CYP1A1* and *CYP1B1* genes can be differentially affected by a pharmacologic agent within an individual cell type. These results may provide a model for investigating the mechanisms that give rise to elevated CYP1B1 expression and E_2 4-hydroxylation in

the process of carcinogenesis of the human breast.

ACKNOWLEDGMENTS

This study was supported by National Institutes of Health grants CA70553 (to D.C.S.) and ES03561 (to J.F.G.) and by Superfund Basic Research grant ES03819. We gratefully acknowledge use of the Wadsworth Center's Biochemistry and Molecular Genetics Core Facilities.

REFERENCES

Abbott BD, Perdew GH, Birnbaum LS (1994): Ah receptor in embryonic mouse palate and effects of TCDD on receptor expression. Toxicol Appl Pharmacol 126:16–25.

Carrier F, Owens RA, Nebert DW, Puga A (1992): Dioxin-dependent activation of murine *Cyp1a-1* gene transcription requires protein kinase C-dependent phosphorylation. Mol Cell Biol 12:1856–1863.

Cavalieri EL, Stack DE, Devanesan PD, Todorovic R, Dwivedy I, Higginbotham S, Johansson SL, Patil KD, Gross ML, Gooden JK, Ramanathan R, Cerny RL, Rogan EG (1997): Molecular origin of cancer: Catechol estrogen-3,4-quinones as endogenous tumor initiators. Proc Natl Acad Sci USA 94:10937–10942.

Chen Y-H, Tukey RH (1996): Protein kinase C modulates regulation of the *CYP1A1* gene by the aryl hydrocarbon receptor. J Biol Chem 271:26261–26266.

Chomczynski P, Sacchi N (1987): Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Anal Biochem 162:156–159.

Christou M, Savas Ü, Spink DC, Gierthy JF, Jefcoate CR (1994): Coexpression of CYP1A1 and a human analog of cytochrome P450-EF in response to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in the human mammary carcinoma-derived MCF-7 cells. Carcinogenesis 15:725–732.

Döhr O, Vogel C, Abel J (1995): Different response of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD)-sensitive genes in human breast cancer MCF-7 and MDA-MB-231 cells. Arch Biochem Biophys 321:405–412.

Fasco MJ, Treanor CP, Spivak S, Figge HL, Kaminsky LS (1995): Quantitative RNA-polymerase chain reaction-DNA analysis by capillary electrophoresis and laser-induced fluorescence. Anal Biochem 224:140–147.

Förster E (1994): An improved general method to generate internal standards for competitive PCR. BioTechniques 16:18–20.

Gierthy JF, Lincoln DW II, Gillespie MB, Seeger JI, Martinez HL, Dickerman HW, Kumar SA (1987): Suppression of estrogen-regulated extracellular tissue plasminogen activator activity of MCF-7 cells by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. Cancer Res 47:6198–6203.

Gierthy JF, Spink BC, Figgee HL, Pentecost BT, Spink DC (1996): Effects of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin, 12-*O*-tetradecanoylphorbol-13-acetate and 17β-estradiol on estrogen receptor regulation in MCF-7 human breast cancer cells. J Cell Biochem 60:173–184.

Hayashi S-I, Okabe-Kado J, Honma Y, Kawajiri K (1995): Expression of Ah receptor (TCDD receptor) during human monocytic differentiation. Carcinogenesis 16:1403–1409.

Hayes CL, Spink DC, Spink BC, Cao JQ, Walker NJ, Sutter TR (1996): 17β-Estradiol hydroxylation catalyzed by human cytochrome P450 1B1. Proc Natl Acad Sci USA 93:9776–9781.

Kress S, Greenlee WF (1997): Cell-specific regulation of human *CYP1A1* and *CYP1B1* genes. Cancer Res 57:1264–1269.

Li JJ, Li SA (1987): Estrogen carcinogenesis in Syrian hamster tissues: Role of metabolism. Fed Proc 46:1858–1863.

Li W, Donat S, Döhr O, Unfried K, Abel J (1994): Ah receptor in different tissues of C57BL/6J and DBA/2J mice: Use of competitive polymerase chain reaction to measure Ah-receptor mRNA expression. Arch Biochem Biophys 315:279–284.

Liehr JG, Ricci MJ (1996): 4-Hydroxylation of estrogens as marker of human mammary tumors. Proc Natl Acad Sci USA 93:3294–3296.

Liehr JG, Roy D (1990): Free radical generation by redox cycling of estrogens. Free Rad Biol Med 8:415–423.

Liehr JG, Fang WF, Sirbasku DA, Ari-Ulubelen A (1986): Carcinogenicity of catechol estrogens in the Syrian hamster. J Steroid Biochem 24:353–356.

Liehr JG, Ricci MJ, Jefcoate CR, Hannigan EV, Hokanson JA, Zhu BT (1995): 4-Hydroxylation of estradiol by human uterine myometrium and myoma microsomes: Implications for the mechanism of uterine tumorigenesis. Proc Natl Acad Sci USA 92:9220–9224.

Marie S, Anderson A, Cresteil T (1988): Transplacental induction of cytochromes P-450IA1 and P-450IA2 by polycyclic aromatic hydrocarbons: TCDD-binding protein level as the rate-limiting step. Carcinogenesis 9:2059–2063.

McKay JA, Melvin WT, Ah-See AK, Ewen SWB, Greenlee WF, Marcus CB, Burke MD, Murray GI (1995): Expression of cytochrome P450 1B1 in breast cancer. FEBS Lett 374: 270–272.

Moore M, Narasimhan TR, Steinberg MA, Wang X, Safe S (1993): Potentiation of *CYP1A1* gene expression in MCF-7 human breast cancer cells cotreated with 2,3,7,8-tetrachlorodibenzo-*p*-dioxin and 12-*O*-tetradecanolylphorbol-13-acetate. Arch Biochem Biophys 305:483–488.

Murray GI, Taylor MC, McFadyen MCE, McKay JA, Greenlee WF, Burke MD, Melvin WT (1997): Tumor-specific expression of cytochrome P450 CYP1B1. Cancer Res 57: 3026–3031.

Nebert DW (1991): Proposed role of drug-metabolizing enzymes: regulation of steady state levels of the ligands that effect growth, homeostasis, differentiation, and neuroendocrine functions. Mol Endocrinol 5:1203–1214.

Okino ST, Pendurthi UR, Tukey RH (1992): Phorbol esters inhibit the dioxin receptor-mediated transcriptional activation of mouse Cyp1a-1 and Cyp1a-2 genes by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. J Biol Chem 267:6991–6998.

Platet N, Prévostel C, Derocq D, Joubert D, Rochefort H, Garcia M (1998): Breast cancer cell invasiveness: Correlation with protein kinase C activity and differential regulation by phorbol ester in estrogen receptor-positive and estrogen receptor-negative cells. Int J Cancer 75:750–756.

Poland A, Glover E (1987): Variation in the molecular mass of the Ah receptor among vertebrate species and strains of rats. Biochem Biophys Res Commun 146:1439–1449.

Pollenz RS (1996): The aryl-hydrocarbon receptor, but not the aryl-hydrocarbon receptor nuclear translocator protein, is rapidly depleted in hepatic and nonhepatic culture cells exposed to 2,3,7,8-tetraclorodibenzo-*p*-dioxin. Mol Pharmacol 49:391–398.

Raunio H, Pelkonen O (1983): Effect of polycyclic aromatic compounds and phorbol esters on ornithine decarboxylase and aryl hydrocarbon hydroxylase activities in mouse liver. Cancer Res 43:782–786.

Ree AH, Landmark BF, Walaas SI, Lahooti H, Eikvar L, Eskild W, Hansson V (1991): Down-regulation of messenger ribonucleic acid and protein levels for estrogen receptors by phorbol esters and calcium in MCF-7 cells. Endocrinology 129:339–334.

Shimada T, Hayes CL, Yamazaki H, Amin S, Hecht SS, Guengerich FP, Sutter TR (1996): Activation of chemically diverse procarcinogens by human cytochrome P450 1B1. Cancer Res 56:2979–2984.

Spink DC, Lincoln DW II, Dickerman HW, Gierthy, JF (1990): 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin causes an extensive alteration of 17β-estradiol metabolism in MCF-7 breast tumor cells. Proc Natl Acad Sci USA 87:6917–6921.

Spink DC, Eugster H-P, Lincoln DW II, Schuetz JD, Schuetz EG, Johnson JA, Kaminsky LS, Gierthy JF (1992): 17β-Estradiol hydroxylation catalyzed by human cytochrome P450 1A1: A comparison of the activities induced by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in MCF-7 cells with those from heterologous expression of the cDNA. Arch Biochem Biophys 293:342–348.

Spink DC, Hayes CL, Young NR, Christou M, Sutter TR, Jefcoate CR, Gierthy JF (1994a): The effects of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin on estrogen metabolism in MCF-7 breast cancer cells: evidence for induction of a novel 17β-estradiol 4-hydroxylase. J Steroid Biochem Mol Biol 51:251–258.

Spink DC, Johnson JA, Connor SP, Aldous KM, Gierthy JF (1994b): Stimulation of 17β-estradiol metabolism in MCF-7 cells by bromochloro- and chloromethyl-substituted dibenzo-*p*-dioxins and dibenzofurans: Correlations with antiestrogenic activity. J Toxicol Environ Health 41:451–466.

Spink DC, Spink BC, Cao JQ, Gierthy JF, Hayes CL, Li Y, Sutter TR (1997): Induction of catechol estrogen metabolism and CYP1B1 in ACHN human renal adenocarcinoma cells. J Steroid Biochem Mol Biol 62:223–232.

Spink DC, Spink BC, Cao JQ, DePasquale JA, Pentecost BT, Fasco MJ, Li Y, Sutter TR (1998): Differential expression of CYP1A1 and CYP1B1 in human breast epithelial cells and breast tumor cells. Carcinogenesis 19:291–298.

Sutter TR, Tang YM, Hayes CL, Wo Y-YP, Jabs EW, Li X, Yin H, Cody CW, Greenlee WF (1994): Complete cDNA sequence of a human dioxin-inducible mRNA identifies a new gene subfamily of cytochrome P450 that maps to chromosome 2. J Biol Chem 269:13092–13099.

Swanson HI, Perdew GH (1993): Half-life of aryl hydrocarbon receptor in Hep 1 cells: Evidence for ligand-dependent alterations in cytosolic receptor. Arch Biochem Biophys 302:167–174.

Thomsen JS, Wang X, Hines RN, Safe S (1994): Restoration of aryl hydrocarbon (Ah) responsiveness in MDA-MB-231 human breast cancer cells by transient expression of the estrogen receptor. Carcinogenesis 15:933–937.

Weisz J, Bui QD, Roy D, Liehr JG (1992): Elevated 4-hydroxylation of estradiol by hamster kidney microsomes: A potential pathway of metabolic activation of estrogens. Endocrinology 131:655–661.

Yager JD, Liehr JG (1996): Molecular mechanisms of estrogen carcinogenesis. Annu Rev Pharmacol Toxicol 36:203–232.