

# Activation of Peroxisome Proliferator-Activated Receptors by Chlorinated Hydrocarbons and Endogenous Steroids

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Trichloroethylene (TCE) and related hydrocarbons constitute an important class of environmental pollutants whose adverse effects on liver, kidney, and other tissues may, in part, be mediated by peroxisome proliferator-activated receptors (PPARs), ligand-activated transcription factors belonging to the steroid receptor superfamily. Activation of PPAR induces a dramatic proliferation of peroxisomes in rodent hepatocytes and ultimately leads to hepatocellular carcinoma. To elucidate the role of PPAR in the pathophysiologic effects of TCE and its metabolites, it is important to understand the mechanisms whereby PPAR is activated both by TCE and endogenous peroxisome proliferators. The investigations summarized in this article *a*) help clarify the mechanism by which TCE and its metabolites induce peroxisome proliferation and *b*) explore the potential role of the adrenal steroid and anticarcinogen dehydroepiandrosterone 3 $\beta$ -sulfate (DHEA-S) as an endogenous PPAR activator. Transient transfection studies have demonstrated that the TCE metabolites trichloroacetate and dichloroacetate both activate PPAR $\alpha$ , a major liver-expressed receptor isoform. TCE itself was inactive when tested over the same concentration range, suggesting that its acidic metabolites mediate the peroxisome proliferative potential of TCE. Although DHEA-S is an active peroxisome proliferator *in vivo*, this steroid does not stimulate *trans*-activation of PPAR $\alpha$  or of two other PPAR isoforms,  $\gamma$  and  $\delta$ /Nuc1, when evaluated in COS-1 cell transfection studies. To test whether PPAR $\alpha$  mediates peroxisomal gene induction by DHEA-S in intact animals, DHEA-S has been administered to mice lacking a functional PPAR $\alpha$  gene. DHEA-S was thus shown to markedly increase hepatic expression of two microsomal P450A proteins associated with the peroxisomal proliferative response in wild-type mice. In contrast, DHEA-S did not induce these hepatic proteins in PPAR $\alpha$ -deficient mice. Thus, despite its unresponsiveness to steroidal peroxisome proliferators in transfection assays, PPAR $\alpha$  is an obligatory mediator of DHEA-S-stimulated hepatic peroxisomal gene induction. DHEA-S, or one of its metabolites, may thus serve as an important endogenous regulator of liver peroxisomal enzyme expression. — *Environ Health Perspect* 106(Suppl 4):983–988 (1998). <http://ehpnet1.niehs.nih.gov/docs/1998/Suppl-4/983-988zhou/abstract.html>

Key words: chlorinated hydrocarbons, trichloroethylene, trichloroacetic acid, DHEA-S, PPAR $\alpha$ , peroxisome proliferators

## Introduction

Trichloroethylene (TCE) is a widely used agent in dry cleaning, paint stripping, and industrial cleaning that is of particular interest to Superfund cleanup efforts. It is a common and persistent environmental pollutant, and has been found in over one-third of hazardous waste sites and in 10% of groundwater sources (1). Exposure to TCE and related chlorinated hydrocarbons is

associated with a number of several adverse health effects, including liver, kidney, and central nervous system toxicity (2). The toxicity of these chemicals appears to be enhanced by their metabolism catalyzed by liver cytochrome P450 (CYP) enzymes, which produce multiple reactive and/or toxic metabolites (3). These metabolites may act, at least in part, via peroxisome

proliferator-activated receptor (PPAR), a ligand-activated transcription factor that belongs to the steroid receptor superfamily (4). Three mammalian PPAR subtypes,  $\alpha$ ,  $\delta$  (or Nuc1), and  $\gamma$ , have been identified. Gene knockout studies in the mouse model demonstrate that PPAR $\alpha$ , which is highly expressed in liver, is responsible for the proliferative effects of chemical peroxisome proliferators such as clofibrate (5). By contrast, PPAR $\delta$ /Nuc1 is expressed in many cell types, whereas PPAR $\gamma$  is abundant in adipose tissues where it plays an important role in adipocyte differentiation (6). The present study investigates the role of TCE and its metabolites in activation of PPAR protein using a transient transfection assay. As reported below, the peroxisome proliferative effects of TCE are mediated by PPAR $\alpha$  via its interactions with TCE's acidic metabolites, trichloroacetic acid (TCA), and dichloroacetic acid (DCA).

To elucidate the role of PPAR in the pathophysiologic effects of TCE and its metabolites, it is additionally important to understand the physiologic effects of PPAR $\alpha$  activation by endogenous regulators. Such information may help identify any synergistic or antagonistic interactions between endogenous peroxisome proliferators and chlorinated hydrocarbons at the level of PPAR receptor activation. One such potential endogenous PPAR activator is dehydroepiandrosterone (DHEA), a naturally occurring adrenal steroid with known peroxisome proliferative potential (7). DHEA is distinguished from other steroids by its chemoprotective properties (8,9). DHEA can also stimulate a dramatic increase in both the size and number of peroxisomes in liver when given to rodents at high doses. This response is accompanied by a substantial increase in peroxisomal  $\beta$ -oxidation and fatty acid-metabolizing CYP4A enzymes (10–14). Moreover, chronic administration of DHEA can lead to hepatocarcinogenesis (15). The cellular mechanism(s) underlying the DHEA-induced peroxisome proliferative effect is poorly understood. In primary rat hepatocytes, DHEA is inactive as a peroxisome proliferator unless it is first metabolized to the corresponding 3 $\beta$ -sulfate (DHEA-S) (16,17). Recent studies on male workers chronically exposed to TCE have shown that increased plasma levels of DHEA-S are associated with years of exposure to TCE, rising from 255 to 718 ng/ml for workers exposed to TCE for less than 3 and greater

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Abbreviations used: ADIOL-S, 5-androstene-3 $\beta$ , 17 $\beta$ -diol 3 $\beta$ -sulfate; CYP, cytochrome P450; DCA, dichloroacetic acid; DHEA, dehydroepiandrosterone; DHEA-S, DHEA 3 $\beta$ -sulfate; DMEM, Dulbecco's modified Eagle's medium; PPAR, peroxisome proliferator-activated receptor; PPAR $\alpha$  knockout mice, (-/-), PPAR $\alpha$  wild-type mice, (+/+); RXR, retinoid X receptor; TCA, trichloroacetic acid; TCE, trichloroethylene; Wy-14,643, pirinixic acid.

than or equal to 7 years, respectively (18). This relationship between DHEA-S and TCE suggests that TCE may disrupt peripheral endocrine function, perhaps through its peroxisome proliferative effects in liver and other tissues. In addition, it is conceivable that TCE may compete with DHEA-S for binding to PPAR, ultimately stimulating an elevation of plasma DHEA-S as a compensatory response. More studies are required to elucidate the mechanism underlying these interactions between TCE and DHEA-S and the potential role of PPAR and peroxisome proliferation in these events.

As is the case for chlorinated hydrocarbons such as TCE, peroxisome proliferation induced by endogenous fatty acids, as well as by structurally diverse hypolipidemic fibrates drugs and other foreign chemicals, is mediated by the  $\alpha$  isoform of PPAR, PPAR $\alpha$  (19). However, as described below, unlike the TCE metabolites trichloroacetate and dichloroacetate, DHEA and DHEA-S fail to activate PPAR $\alpha$  in transient cotransfection assays. It is possible that DHEA and/or DHEA-S might mediate their effects through other related receptors, specifically, PPAR $\gamma$  (20) or PPAR $\delta$ /Nuc1 (21). Alternatively, the *in vitro* transfection systems used to test for DHEA and/or DHEA-S activation of PPAR $\alpha$  may be insufficiently sensitive to detect weak activation by the steroid or may lack metabolic capacity or other key factors present only in the intact animal. Several of these possibilities have been examined recently (22), along with the role of PPAR $\alpha$  in DHEA-S-induced peroxisome proliferation *in vivo* using a mouse line that lacks the PPAR $\alpha$  receptor (5) and its associated pleiotropic response to peroxisome proliferators. These studies are summarized below. The results establish that despite its apparent inactivity *in vitro*, PPAR $\alpha$  mediates the *in vivo* effects of DHEA-S on peroxisomal proliferation.

## Materials and Methods

### Plasmids

Reporter plasmid pLucA6-880, containing 880 nts (nucleotides) of 5'-flanking DNA of the rabbit *CYP4A6* gene cloned into p19Luc, and the mouse PPAR $\alpha$  expression plasmids pCMV-PPAR $\alpha$  and pCMV-PPAR $\alpha$ -G (23) were kindly provided by E. Johnson (The Scripps Research Institute, La Jolla, CA). A plasmid expressing full-length human PPAR $\delta$ /Nuc1 cloned into pJ3omega (21) was kindly provided by A.

Schmidt (Merck Research Labs, West Point, PA). Mouse PPAR $\gamma$  cloned into pSV-Sport1 (24) was provided by J. Reddy (Northwestern University Medical School, Chicago, IL) and mouse RXR $\alpha$  expression plasmid pCMX-mRXR $\alpha$  (25) was provided by R. Evans (Salk Institute, San Diego, CA).  $\beta$ -Galactosidase expression plasmid (pSV- $\beta$ -gal) was purchased from Promega (Madison, WI).

### Transfection Studies

Transfection of COS-1 cells grown in 12-well tissue culture plates was carried out by a calcium phosphate precipitation method. After transfection, cells were incubated in Dulbecco's modified Eagle's medium (DMEM) containing 10% charcoal-stripped, delipidated bovine calf serum. Transfections were performed as described elsewhere (22) using a  $\beta$ -galactosidase plasmid as an internal control. Chlorinated hydrocarbons, including TCE, TCA, and DCA, were purchased from Aldrich Chemical Co. (Milwaukee, WI) and were dissolved in DMEM before administered to cells. Potential PPAR activators, including Wy-14,643, DHEA-S, DHEA, and ADIOL were purchased from Sigma Chemical Co. (St. Louis, MO), each diluted from a 1000-fold stock in dimethyl sulfoxide. Chemicals were added to the cells in fresh media 24 hr after transfection at the concentrations indicated. Forty-eight hours after initiating the transfection, cells were washed twice with cold phosphate-buffered saline, then dissolved by incubation for 15 min at 4°C in lysis solution (100 mM KPi, pH 7.8, 0.2% Triton X-100, with 1 mM dithiothreitol added prior to use; 80  $\mu$ l/well). The cell extract was then scraped and transferred to a centrifuge tube for removal of insoluble cell debris in an Eppendorf centrifuge. Luciferase and  $\beta$ -galactosidase activities were measured. Luciferase activity values were normalized for transfection efficiency using  $\beta$ -galactosidase activity values determined from the same preparation of cell lysate.

### PPAR Knockout Mice

Male PPAR $\alpha$  (-/-) mice or (+/+) ( $F_3$  homozygotes or wild-type; hybrids of C57BL/6N  $\times$  ISV129 genetic background; 10–12 weeks of age) (5) were injected with either DHEA-S or clofibrate (Sigma) at 15 mg/100 g body weight or corn oil (vehicle control) for 4 consecutive days by intraperitoneal injection (22). Twenty-four hours after the final injection, mice were killed by carbon dioxide asphyxiation, and

the liver and kidneys were removed and used for isolation of microsomes.

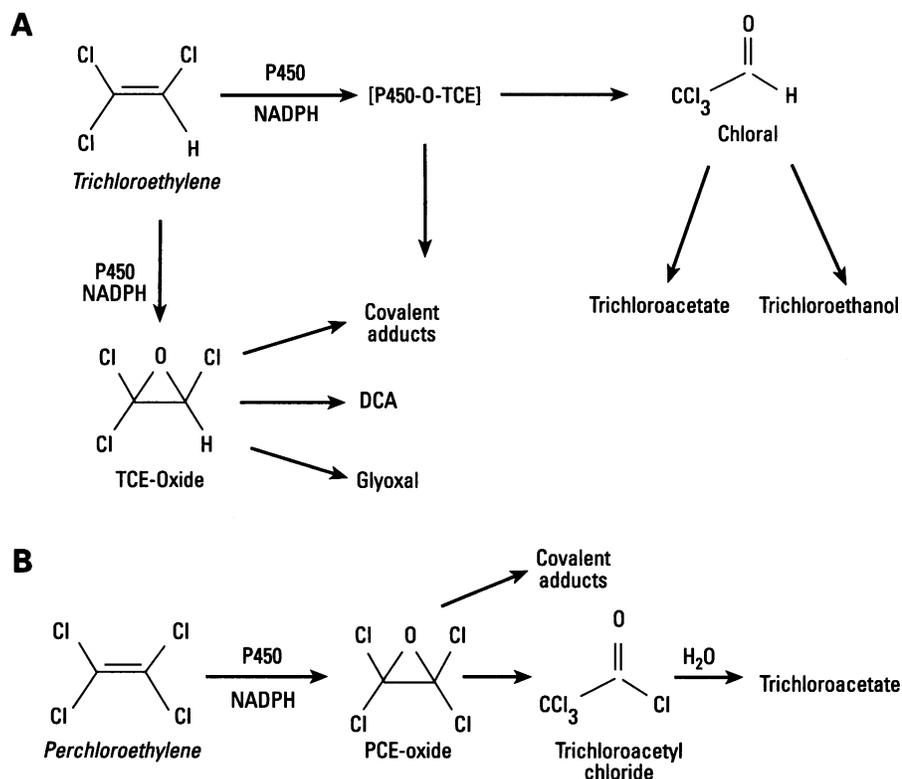
### Analysis of Microsomal CYP4A Protein Expression

Liver microsomes prepared from frozen tissues by differential centrifugation were analyzed by Western blotting using polyclonal anti-rat CYP4A antibody raised to a di(2-ethylhexyl)phthalate-inducible rat liver CYP4A protein related to CYP4A1. This antibody has been characterized elsewhere (27) and was provided by R.T. Okita (Washington State University, Pullman, WA).

## Results

### Chlorinated Hydrocarbons and Peroxisome Proliferation

Rodent bioassays establish that TCE is a complete hepatocarcinogen, with chronic exposure to TCE leading to hepatocellular carcinoma development (28,29). The hepatotoxicity and carcinogenicity of TCE appears to be related directly to the extent of its oxidative metabolism, which is primarily catalyzed by liver CYP enzymes and yields multiple reactive and toxic metabolites (Figure 1). At least some of these active metabolites may achieve their deleterious effects via a mechanism that involves peroxisome proliferation (28). Peroxisome proliferation is a trophic phenomenon in the liver, originally described after administration of the hypolipidemic drug clofibrate to rodents (30). This proliferative response is characterized in the short term by a dramatic increase in both the size and number of peroxisomes. It is also associated with upregulation of peroxisomal fatty acid  $\beta$ -oxidation enzymes and microsomal P4504A fatty acid hydroxylase enzymes as well as increased cell differentiation and liver weight gain. Chronic exposure to peroxisome proliferators leads to hepatocellular carcinoma. A broad spectrum of structurally diverse compounds, including certain hypolipidemic drugs, herbicides, industrial solvents, and the adrenal steroid DHEA, has been shown to induce peroxisome proliferation. These peroxisome proliferators stimulate liver growth and tumor formation by a nongenotoxic mechanism, i.e., one that does not involve DNA damage caused by the peroxisome proliferators or their metabolites (31). PPAR, a ligand-activated transcription factor and a member of the steroid receptor superfamily, has been shown to be activated by diverse peroxisome proliferators and can



**Figure 1.** Pathways of P450-dependent metabolism of TCE and PCE.

thus mediate their peroxisome proliferative effects (19).

### Activation of PPAR $\alpha$ by Chlorinated Hydrocarbons

Trichloroacetic acid and dichloroacetic acid are secondary metabolites of TCE (32). TCA, in particular, has been implicated as a key hepatocarcinogenic metabolite of TCE and is believed to act by inducing peroxisome proliferation (33). Transient *trans*-activation assays using chimeric receptors (ER/PPAR $\alpha$  and GR/PPAR $\alpha$ ) containing a PPAR $\alpha$  transactivation domain suggest that TCA may be a weak activator of PPAR $\alpha$  (19). To investigate the responsiveness of PPAR to activation by TCE and its acidic metabolites, TCA and DCA, COS-1 cells were cotransfected with a PPAR expression plasmid, pCMV-mPPAR $\alpha$ , together with a reporter plasmid containing a peroxisome proliferator response element, pLuc4A6-880 (23). As shown in Table 1, treatment of the transfected COS-1 cells with TCA and DCA for 24 hr resulted in the activation of a luciferase reporter gene. This activation was not apparent at 0.1 mM TCA or 0.1 mM DCA, but was readily seen at the two higher concentrations tested, 1 and 5 mM. Activations of 16- and 10-fold,

respectively, were observed with TCA and DCA at concentrations of 5 mM. When tested over the same concentration range, TCE alone did not substantially activate reporter gene expression (Table 1). These results indicate that the PPAR $\alpha$ -dependent effects of TCE on gene expression most likely proceed through its oxidative metabolites TCA and DCA. The specific P450 enzymes that catalyze the oxidative metabolism of TCE, and that ultimately yield TCA and DCA, may thus play a critical role in the activation of TCE to metabolites that contribute to its deleterious effects on liver, kidney, and perhaps other tissues.

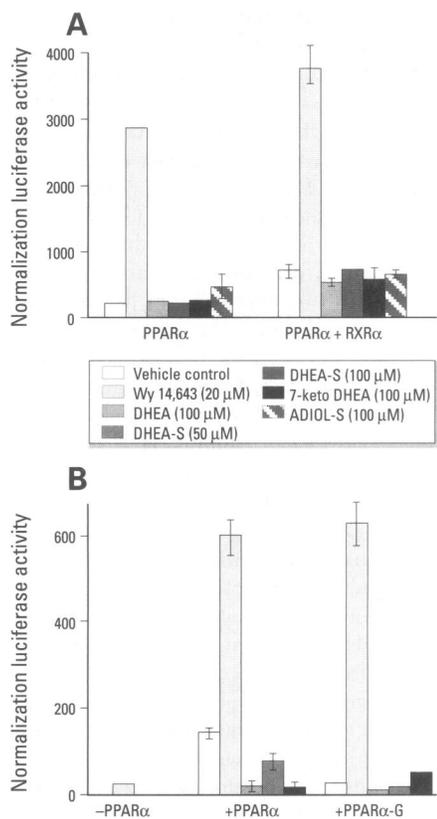
### DHEA-S-Induced CYP4A Induction *in Vivo*

Dehydroepiandrosterone is a naturally occurring steroid hormone that has various beneficial effects on rodents, including antidiabetic, anticarcinogenic, and antiobesity effects. DHEA has been characterized as a peroxisome proliferator (7). At pharmacologic doses, DHEA induces peroxisome proliferation, with an increased expression of peroxisomal  $\beta$ -oxidation enzymes and some other enzymes involved in lipid metabolism such as microsomal CYP4A enzymes. Like other peroxisome proliferators, DHEA can induce hepatocarcinogenesis when administered to rodents at moderate to high doses. The apparent peroxisome proliferative effect of DHEA in intact animals and its ineffectiveness at inducing peroxisomal gene expression in cultured hepatocytes (16,17) suggest that DHEA undergoes metabolism *in vivo* to an active derivative that mediates the peroxisome proliferative response. The finding that the sulfate of DHEA, DHEA-S, is an active inducer of peroxisomal enzyme and CYP4A expression in hepatocyte culture (16) raised the possibility that DHEA sulfation, catalyzed by liver sulfotransferase enzymes, is a prerequisite for DHEA to attain its peroxisome proliferative effects. To investigate this possibility, studies were conducted to determine whether DHEA-S is preferred to DHEA with respect to CYP4A induction *in vivo* (22). DHEA-S given at a low dose (10 mg/kg daily for 4 days) was found to be substantially more active than DHEA with respect to liver CYP4A3 mRNA induction. This finding is consistent with the observation that acetaminophen, an inhibitor of sulfate conjugation, reduces the peroxisomal  $\beta$ -oxidation activity induced by DHEA, but does not affect the activity induced by DHEA-S and clofibrate (17). By contrast, at a higher dose of steroid (60 mg/kg), DHEA and DHEA-S were equally active at inducing a

**Table 1.** Activation of PPAR $\alpha$  by chlorinated hydrocarbons. Normalized luciferase activity, fold increase.

Concentration, mM	Trichloroacetic acid	Dichloroacetic acid	Trichloroethylene
Control	1.00	1.00	1.00
0.1	1.06 $\pm$ 0.11	1.31 $\pm$ 0.11	1.27
1.0	8.14 $\pm$ 1.56	4.18 $\pm$ 0.37	1.74 $\pm$ 0.54
5.0	16.54 $\pm$ 0.04	10.94 $\pm$ 1.24	1.72 $\pm$ 0.25

Cotransfection of PPAR $\alpha$  expression plasmid with a P4504A6 promoter-luciferase reporter construct was carried out in COS-1 monkey kidney cells using a calcium phosphate precipitation method. Cells were treated with either TCA, DCA, or TCE, as indicated, for a 24-hr period beginning 24 hr after washing of the cells to remove the calcium phosphate DNA precipitate. Luciferase reporter activity of cell extracts was then determined and the data were normalized to a  $\beta$ -galactosidase reporter (pSV- $\beta$ -gal) as an internal standard. Data shown are mean  $\pm$  range for duplicate determinations.



**Figure 2.** Activation of PPAR $\alpha$  by peroxisome proliferators: Cotransfection of PPAR $\alpha$  or PPAR $\alpha$ -G expression plasmids with P4504A6 promoter-luciferase reporter construct (4A6-Luc) in the presence or absence of an RXR $\alpha$  expression plasmid was carried out in COS-1 cells using calcium phosphate precipitation method. Cells were treated with the indicated PPAR activators for 24 hr. Luciferase reporter activity was then determined and the data normalized to a  $\beta$ -galactosidase reporter (pSV- $\beta$ -gal) as an internal standard. Data shown are mean  $\pm$  range ( $n=2$ ) or mean  $\pm$  SD ( $n=3$ ) based on replicate independent samples. COS-1 cells exhibited a low level of endogenous receptor activity (-PPAR $\alpha$ , first bar in *B*), but a comparatively high level of endogenous PPAR $\alpha$  activator (PPAR $\alpha$  + 4A6Luc + vehicle control). This endogenous activator activity was greatly reduced when using the mouse PPAR $\alpha$ -G mutant (*B*). Both receptors were strongly activated by Wy-14,643 but not by DHEA-S, DHEA, 7-keto DHEA, or ADIOL 3 $\beta$ -sulfate.

peroxisome proliferative response (22). The equal effectiveness of DHEA and DHEA-S at the higher dose is presumably due to the efficient sulfation of DHEA *in vivo*, in a reaction catalyzed by liver sulfotransferases.

#### **trans-Activation of PPAR by DHEA-S and Related Steroids**

Although many foreign chemical peroxisome proliferators can activate PPAR $\alpha$  to initiate pathophysiologic events, the physiologic effects of PPAR $\alpha$  activation are likely to

involve endogenous regulators that may serve to modulate PPAR $\alpha$  activity. Characterization of the physiologic role of PPAR $\alpha$  in responding to these endogenous activators is thus critical for a full understanding of the pathophysiologic effects of foreign peroxisome proliferators, including TCE and its metabolites. Endogenous PPAR $\alpha$  activators derived from fatty acids and their metabolites have been described and include linoleic acids, polyunsaturated fatty acids, and eicosanoids (34–36). By contrast, steroidal activators of PPAR have not been identified. In view of its peroxisomal proliferative effects *in vivo*, DHEA-S is a good candidate for an endogenous steroidal PPAR $\alpha$  activator. Studies were therefore conducted to investigate the role of PPAR $\alpha$  in DHEA-S-activated peroxisome proliferation using transient transfection methods (22). Unlike the prototypic foreign chemical peroxisome proliferator Wy-14,643, which can induce luciferase reporter activity by 15-fold after 24-hr treatment of PPAR $\alpha$ -transfected COS-1 cells, DHEA-S was unable to induce reporter activity. DHEA, and the related steroids 7-keto-DHEA and ADIOL-S were also inactive in terms of induction of PPAR $\alpha$ -stimulated reporter activity (Figure 2A).

Retinoid X receptor (RXR) is a common partner for many steroid receptors. RXR forms a heterodimer with PPAR and this heterodimerization enhanced PPAR-DNA binding and transcriptional activation activity (37). To investigate whether RXR is required for DHEA-S-induced PPAR activation, a mouse RXR $\alpha$  expression plasmid was cotransfected with pCMV-PPAR $\alpha$ . As shown in Figure 2A, basal luciferase reporter activity was increased 3-fold in cells transfected with RXR $\alpha$ . However, no further increase in activity was detected after treatment of the transfected cells with DHEA, 7-keto DHEA, DHEA-S, or ADIOL-S.

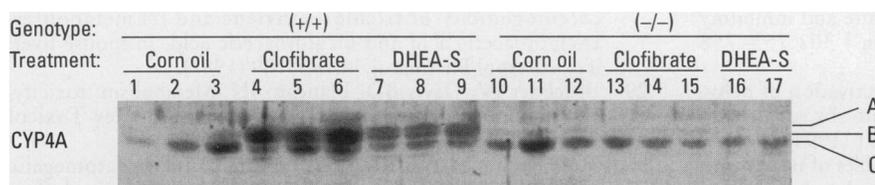
Transfection of PPAR $\alpha$  expression plasmid results in a substantial increase in basal luciferase reporter activity in the absence of peroxisome proliferator treatment, as seen by comparing the -PPAR $\alpha$  sample with the +PPAR $\alpha$ /vehicle control in Figure 2B. This finding suggests the existence of endogenous activator(s) of PPAR $\alpha$  in COS-1 cells. Similar results have been reported by others (23). PPAR $\alpha$ -G, a PPAR $\alpha$  mutant that has a Glu<sup>282</sup> to Gly substitution, can substantially lower the basal activation while it remains sensitive to peroxisome proliferator activation (38). Given this potentially greater sensitivity for

detection of a weak peroxisome proliferative response using this mutant receptor, PPAR $\alpha$ -G was tested in transfection studies to examine whether DHEA-S can induce a low activation of PPAR. Figure 2B shows that PPAR $\alpha$ -G transfection results in a 6-fold decrease in basal PPAR activation when compared to wild-type PPAR $\alpha$ , and its activity was induced 30-fold after Wy-14,643 treatment in the experiment shown. However, no increase in reporter gene activity could be detected in cells treated with DHEA, 7-keto DHEA, DHEA-S, or ADIOL-S, either in the absence (Figure 2B; data not shown) or in the presence of cotransfected RXR (data not shown).

To address the possibility that other PPAR subtypes may mediate DHEA-S-dependent peroxisome proliferator responses, cotransfection experiments have been carried out using PPAR $\gamma$  and PPAR $\delta$ /Nuc1 expression plasmids in the presence of cotransfected RXR $\alpha$ . PPAR $\gamma$  and PPAR $\delta$ /Nuc1 were found to be weakly activated by high concentrations of Wy-14,643 (100  $\mu$ M), in agreement with a previous report (39). However, DHEA, DHEA-S, and ADIOL-S did not induce significant responses from PPAR $\gamma$  or PPAR $\delta$ /Nuc1 (22). Therefore, despite the fact that DHEA-S is an active peroxisome proliferator *in vivo* and in primary rat hepatocytes, it is apparently inactive with respect to PPAR activation in transient transactivation assays using cultured cells that respond to a wide range of other PPAR activators and peroxisome proliferators.

#### **Influence of PPAR $\alpha$ Gene Knockout on DHEA-S-Induced Peroxisome Proliferation in Liver**

To probe the role of PPAR $\alpha$  for a DHEA-S-stimulated peroxisome proliferative response *in vivo*, PPAR $\alpha$  knockout mice and wild-type mice (5) were tested for their responsiveness to DHEA-S-induced peroxisome proliferation. As we recently reported (22), Western blot analysis of liver microsomal CYP4A revealed two CYP4A proteins that were highly inducible in livers of PPAR $\alpha$  wild-type mice [PPAR $\alpha$  (+/+)] mice when treated with DHEA-S and clofibrate. In contrast, those same CYP4A proteins were not induced by either clofibrate or DHEA-S injection in PPAR $\alpha$  knockout mice [PPAR $\alpha$  (-/-)] mice liver. A constitutively expressed CYP4A immunoreactive protein of slightly lower apparent molecular weight was also detected (Figure 3, band C), but its level was unaffected by either peroxisome proliferator or by the PPAR $\alpha$  knockout phenotype



**Figure 3.** Evaluation of DHEA-S induction of liver CYP4A proteins expression in PPAR $\alpha$  (+/+) and PPAR $\alpha$  (-/-) mice. Western blot analysis of liver microsomes P4504A protein using anti-4A antibody. Microsomes were prepared from liver samples corresponding to the samples shown in A. P450 4A-immunoreactive proteins marked A and B are induced by clofibrate and DHEA-S, but only in PPAR $\alpha$  (+/+) mice, whereas band C corresponds to a constitutively expressed protein that is unaffected by the gene knockout.

(Figure 3). These results are consistent with Northern blot results showing strong increases in the hepatic mRNAs encoding CYP4A1, CYP4A3, acyl-CoA-oxidase, bifunctional enzyme, and 3-ketoacyl-CoA thiolase in PPAR $\alpha$  (+/+) mice but not PPAR $\alpha$  (-/-) mice after DHEA-S and clofibrate treatment (22).

Thus, although DHEA-S is inactive with respect to PPAR activation in transient *trans*-activation assays in COS-1 cells, experiments carried out using a PPAR $\alpha$  gene knockout mouse model demonstrate that PPAR $\alpha$  is required for DHEA-S induction of hepatic peroxisome proliferation responses. These studies also indicate

that the peroxisome proliferative response of DHEA-S is not mediated by two other PPAR forms, PPAR $\gamma$  and PPAR $\delta$ /Nuc1, despite the presence of the latter nuclear receptor at a significant level in liver tissue (20,24). Several mechanisms could explain the discrepancy between the findings from the *in vivo* study and transient cell transfection experiments: a) Other factors that may be necessary for DHEA-S induction of peroxisome proliferation *in vivo* may be absent from the *in vitro* PPAR *trans*-activation system. b) DHEA-S might act in liver or other tissues to stimulate production of another endogenous chemical that serves as a proximal PPAR $\alpha$

activator. c) The entry of DHEA-S into cells may require a specific plasma membrane transporter that is known to be present in hepatocytes (40), but may be absent in COS-1 and other cell types used for PPAR transfection studies. Finally, d) DHEA-S may be converted to an activated metabolite by a metabolic process which occurs in hepatocytes but not in the cell lines used for transfection studies.

In conclusion, the findings summarized in this report establish that oxidized metabolites of TCE and other chlorinated hydrocarbons, including TCA and DCA, activate mouse PPAR $\alpha$ . *In vivo* experiments further establish that PPAR $\alpha$  is an obligatory mediator of the hepatic gene induction effects of the endogenous steroidal peroxisome proliferator DHEA-S. Further investigation will be necessary to elucidate any interactions that may occur between DHEA-S and chlorinated hydrocarbons at the level of receptor activation, and to determine whether this potential for DHEA-S and its metabolites to serve as physiologic modulators of liver fatty acid metabolism and peroxisomal enzyme expression contributes to the anticarcinogenic and other beneficial chemoprotective properties of this intriguing class of endogenous steroids.

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