The Steroid Hormone Dehydroepiandrosterone Inhibits *CYP1A1* Expression *in Vitro* By a Post-transcriptional Mechanism*

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Henry P. Ciolino‡ and Grace Chao Yeh

From the Cellular Defense and Carcinogenesis Section, Basic Research Laboratory, Division of Basic Sciences, NCI-Frederick Cancer Research and Development Center, National Institutes of Health, Frederick, Maryland 21702-1201

The adrenal steroid hormone dehydroepiandrosterone (DHEA) is a potent inhibitor of mammary carcinogenesis induced by polycyclic aromatic hydrocarbons (PAH), though its mechanism is unclear. We examined the effect of DHEA on the expression of the carcinogen-activating enzyme cytochrome P450 1A1 (*CYP1A1*) in MCF-7 human breast epithelial carcinoma cells. DHEA inhibited the increase in *CYP1A1* enzyme activity that occurs when MCF-7 cells are exposed to the PAH dimethylbenzanthracene (DMBA) or 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). However, DHEA did not directly inhibit enzyme activity as it had no effect when added to the cells after induction by DMBA or TCDD. We observed that the increase of *CYP1A1* mRNA in MCF-7 cells caused by DMBA or TCDD was inhibited by DHEA in a concentration-dependent manner. However, DHEA did not inhibit *CYP1A1* promoter-driven transcription, indicating that it did not affect the aryl hydrocarbon receptor, which regulates transcription of the *CYP1A1* gene. Actinomycin D chase experiments showed that DHEA caused a time- and concentration-dependent decrease in *CYP1A1* mRNA levels, indicating that DHEA inhibits *CYP1A1* expression by decreasing *CYP1A1* mRNA stability. These data demonstrate that DHEA inhibits PAH-induced *CYP1A1* mRNA expression and enzyme activity *in vitro* by a post-transcriptional mechanism. This regulation of the expression of carcinogen-activating enzymes may be responsible for the chemopreventive activity of DHEA and may be one of its physiologic functions in *vivo*.

Dehydroepiandrosterone (DHEA)1 and its sulfated form (DHEA-S) are the major secretory products of the adrenal cortex and are the most abundant steroids in humans, with circulating levels in young adults of 5–7 μM (1). Other than their role as precursors of sex steroid hormones (2, 3), their physiologic functions remain unclear. DHEA and/or DHEA-S have been associated with a number of beneficial effects in humans (4) including decreased cardiovascular disease (5), weight loss (6), reduced serum cholesterol (7), and activation of the immune system (8). Thus, the inexorable decline in circulating levels of these hormones that occurs with age, to 5% of peak values by the ninth decade (1, 9), is of substantial concern.

DHEA has also been shown to have considerable chemopreventive activity toward cancer. A significant body of evidence suggests that DHEA may protect against certain cancers (10, 11). In animal models, DHEA has been shown to inhibit both spontaneous (12) and chemically induced (13, 14) carcinogenesis in rodents. Specifically, DHEA inhibits both skin (15, 16) and mammary tumorogenesis (17–19) caused by dimethylbenzanthracene (DMBA). DMBA is a polycyclic aromatic hydrocarbon (PAH), a class of carcinogen that requires activation to genotoxic metabolites that bind DNA. DHEA has been shown to inhibit DMBA activation *in vitro* (20) and DMBA-DNA binding *in vivo* (21). The activation of PAH is catalyzed by the cytochrome P450 1A and 1B families (22, 23), which require NADPH as a cofactor. Because DHEA is a potent uncompetitive inhibitor of glucose-6-phosphate dehydrogenase *in vitro* (24) (the rate-limiting enzyme in the pentose phosphate pathway that generates NADPH), the inhibition of DMBA-induced carcinogenesis by DHEA has been ascribed to decreased activation by CYP enzymes because of a lack of NADPH. However, inhibition of glucose-6-phosphate dehydrogenase and depletion of NADPH pools *in vivo* have not been demonstrated (12, 25, 26). Indeed, inhibition of cellular glucose-6-phosphate dehydrogenase *in vitro* occurs only at very high DHEA concentrations (27, 28). Furthermore, other enzymes such as isocitrate dehydrogenase and malic enzyme also produce NADPH (25). Thus, the mechanism of DHEA’s chemopreventive activity toward DMBA is unknown.

In the current work we examine an alternative hypothesis to account for the inhibition of DMBA-induced carcinogenesis by DHEA. DMBA, in common with other PAHs, induces the expression of the carcinogen-activating enzyme *CYP1A1* (29). We report that DHEA, but not DHEA-S, inhibits the expression of *CYP1A1* *in vitro* by affecting the stability of *CYP1A1* mRNA, thereby preventing the induction of *CYP1A1* enzyme activity by carcinogens such as DMBA.

**EXPERIMENTAL PROCEDURES**

**Materials**—MCF-7 cells were from the American Type Culture Collection (Manassas, VA). RPMI 1640, glutamine, fetal bovine serum, trypsin/EDTA, phosphate-buffered saline, and Tris borate buffer were from Biofluids (Rockville, MD). Actinomycin D, α-naphthoflavone (α-NF), DHEA, DHEA-S, DMBA, EDTA, ethoxyresorufin, resorufin, Tris, HCl, and dimethyl sulfoxide (Me2SO) were from Sigma. [32P]dATP was from NEN Life Science Products. 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) from the Midwest Research Institute (Kansas City, MO). RT-PCR was performed with an Omniscript kit from Qiagen (Valencia, CA). Tris borate gels, Tris borate running buffer, and high density sample buffer were from Novex (San Diego, CA). Primers for glyceraldehyde-3-phosphate dehydrogenase (GAPDH) RT-PCR and the β-galactosidase-containing reporter vector were from CLONTECH (Palo Alto, CA).
group mean values were determined by a one-factor analysis of variance. The transcription was determined as described previously (32).

CYP1A1 mRNA stability; 24 cycles otherwise) and GAPDH (17 cycles) were performed as described previously (32). cDNA was synthesized from 2 g of total RNA using an Omniscript RT-PCR kit as instructed. A cycle number that fell within the exponential range of response for both CYP1A1 (27 cycles for the determination of basal CYP1A1 mRNA and CYP1A1 mRNA stability; 24 cycles otherwise) and GAPDH (17 cycles) was used.

Transient Transfections—CYP1A1 promoter-controlled CAT transcrip-
tion was determined as described previously (32).

Statistical Analyses—Statistical analyses were performed using Sta-
StatView statistical analysis software (SAS Institute). Differences between
group mean values were determined by a one-factor analysis of variance

TABLE I

| Inducer    | Competitor          | Activity (pmol/min/100,000 cells) |
|------------|---------------------|----------------------------------|
| DMBA       | None (Me2SO)        | 1.28 ± 0.04                      |
| DMBA       | + α-Naphthoflavone  | 0.27 ± 0.04                      |
| DMBA       | + DHEA              | 1.17 ± 0.06                      |
| DMBA       | + 1 μM DHEA-S       | 14.59 ± 0.88                     |
| DMBA       | + DHEA              | 1.17 ± 0.06                      |
| TCDD       | None (Me2SO)        | 14.59 ± 0.88                     |
| TCDD       | + α-Naphthoflavone  | 6.38 ± 0.33                      |
| TCDD       | + DHEA              | 14.82 ± 0.41                     |

RESULTS

DHEA Inhibits Carcinogen-induced CYP1A1 Enzyme Activity—Incubation of MCF-7 cells with 1 μM DMBA for 24 h caused an increase of CYP1A1 activity from undetectable levels in untreated cells to a specific activity of 1.52 ± 0.16 pmol/min/100,000 cells as measured in intact cells using the EROD assay. In cells co-incubated with DMBA and DHEA there was a concentration-dependent decrease in EROD activity, with a concentration of approximately 100 nM at which 50% inhibition (IC50) occurred (Fig. 1A). The sulfur-conjugated form of DHEA-S had no effect on DMBA-induced EROD activity (Fig. 1A).

Treatment of MCF-7 cells with the potent AHR ligand TCDD caused an induction of EROD activity to 15.06 ± 0.89 pmol/min/100,000 cells. This induction was also inhibited by DHEA, but not by DHEA-S, with an IC50 of approximately 1 μM (Fig. 1B).

MCF-7 cells were incubated with DMBA or TCDD to induce CYP1A1 enzyme activity and postincubated with DHEA to
Determine whether the effect of DHEA on CYP1A1 activity results from a direct inhibitory action on the enzyme. As shown in Table I, DHEA had no effect on cellular CYP1A1 activity when added after induction by DMBA or TCDD, whereas the direct, non-competitive CYP1A1 enzyme inhibitor α-NF caused a significant inhibition of enzyme activity. DHEA was also unable to inhibit CYP1A1 enzyme activity in the microsomal fraction isolated from TCDD-treated cells, whereas α-NF completely abolished CYP1A1 activity (data not shown).

**DHEA Inhibits CYP1A1 mRNA Expression**—MCF-7 cells were treated with or without DMBA and DHEA, and the amount of CYP1A1 mRNA was determined by RT-PCR. As shown in Fig. 2, exposure of the cells to DMBA for 6 h resulted in a 5-fold increase in CYP1A1 mRNA compared with untreated cells. In the presence of DHEA, this induction was diminished in a concentration-dependent manner to an approximately 2-fold increase in cells co-treated with 1 μM DHEA (Fig. 2). Treatment of MCF-7 cells with TCDD resulted in an 11-fold increase in CYP1A1 mRNA compared with untreated cells. Co-incubation with increasing concentrations of DHEA had no affect on transcription. Resveratrol, a dietary polyphenolic compound that is a potent inhibitor of AHR activity and CYP1A1 transcription (32), completely inhibited CAT transcription.

**DHEA Decreases the Stability of CYP1A1 mRNA**—The effect of DHEA on the stability of CYP1A1 mRNA was assessed by RT-PCR. Following treatment with DMBA to induce CYP1A1 expression, MCF-7 cells were treated with actinomycin D at 5 μg/ml, a concentration at which CYP1A1 transcription is com-

**FIG. 4.** Lack of effect of DHEA-S on DMBA- or TCDD-induced CYP1A1 mRNA expression. MCF-7 cells were treated with 1 μM DMBA or 1 nM TCDD in the presence of Me₂SO (controls) or 5 μM DHEA-S for 6 h. CYP1A1 and GAPDH (G-3-PDH) mRNA were determined by RT-PCR. There was no significant difference in CYP1A1 mRNA in the presence of DHEA-S.

**FIG. 5.** Inhibition of basal CYP1A1 mRNA levels by DHEA. MCF-7 cells were treated with the indicated concentrations of DHEA for 24 h. CYP1A1 and GAPDH (G-3-PDH) mRNA were determined by RT-PCR. n = 3 ± S.E. There was a significant decrease in basal CYP1A1 mRNA in cells treated with all concentrations of DHEA compared with controls (p < 0.05).

**FIG. 3.** Inhibition of TCDD-induced CYP1A1 mRNA expression by DHEA. MCF-7 cells were treated with 1 nM TCDD in the presence of the indicated concentrations of DHEA for 6 h. CYP1A1 and GAPDH (G-3-PDH) mRNA were determined by RT-PCR. CYP1A1 mRNA was normalized to GAPDH mRNA. n = 3 ± S.E. There was a significant decrease in CYP1A1 mRNA in cells treated with all concentrations of DHEA compared with controls (p < 0.05).
As shown in Fig. 7, CYP1A1 mRNA levels were reduced in a concentration-dependent manner in DHEA-treated cells compared with controls. The presence of DHEA caused an increase in the rate of degradation of CYP1A1 mRNA compared with controls (Fig. 8). The half-life of CYP1A1 mRNA decreased from approximately 7 h in controls to less than 2 h in DHEA-treated cells.

**DISCUSSION**

Among the best characterized molecular responses to PAHs is the induction of the gene CYP1A1, which encodes the carcinogen-activating enzyme CYP1A1 (34). Inhibition of carcinogen-activating enzymes, either by inhibiting enzyme activity or expression, is an important strategy in cancer chemoprevention (35). In the present study we tested the hypothesis that the established chemopreventive activity of DHEA toward aryl hydrocarbon-induced carcinogenesis may be due, in part, to its effects on the induction of CYP1A1 by PAHs. We used MCF-7 human breast epithelial carcinoma cells as a model system in these experiments because they are derived from the target tissue of DMBA, which is primarily a mammary carcinogen, and because CYP1A1 expression has been extensively characterized in this cell line (36, 37).

DHEA causes a concentration-dependent decrease in DMBA- or TCDD-induced CYP1A1 enzyme activity (Fig. 1, A and B). DHEA was more effective at inhibiting DMBA-induced enzyme activity than TCDD-induced activity, possibly because of the much higher levels of induction caused by TCDD. With either ligand, the IC$_{50}$ of DHEA was well below its physiologic concentrations (1), suggesting that DHEA could possibly exert a similar inhibitory effect in vivo. Although decreased carcinogen-activating enzyme activity has been previously suggested to explain the chemopreventive activity of DHEA toward aryl hydrocarbon-induced carcinogenesis may be due, in part, to its effects on the induction of CYP1A1 by PAHs. We used MCF-7 human breast epithelial carcinoma cells as a model system in these experiments because they are derived from the target tissue of DMBA, which is primarily a mammary carcinogen, and because CYP1A1 expression has been extensively characterized in this cell line (36, 37).

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inhibiting enzyme activity. However, as shown in Table I, DHEA had no direct effect on CYP1A1 enzyme activity. Because CYP1A1 activity was reduced without direct enzyme inhibition, we investigated the effect of DHEA on CYP1A1 expression. DHEA inhibited the increase in CYP1A1 mRNA caused by DMBA (Fig. 2) or TCDD. Consistent with the EROD data, DHEA was more effective at lower concentrations (<1 μM) in preventing the increase in CYP1A1 mRNA caused by DMBA than the increase caused by TCDD. In agreement with the enzyme data, DHEA-S had no effect on CYP1A1 mRNA induction caused by either ligand (Fig. 4). Thus, the inhibitory effect of DHEA on CYP1A1 enzyme activity is the result of inhibition of CYP1A1 expression.

CYP1A1 expression is known to be regulated at the transcriptional level by the AHR, which, when activated by ligands such as DMBA or TCDD, acts as a transcription factor by binding to the CYP1A1 promoter and up-regulating transcription. As shown in Fig. 6, treatment of transfected cells with DMBA or TCDD caused a 4- or 7-fold increase, respectively, in CYP1A1 mRNA levels in the absence of treatment with AHR ligands (Fig. 5), suggesting the DHEA operates by a mechanism other than by inhibiting AHR activity. This was confirmed by ligand binding and gel shift assays, which showed that DHEA had no effect on the binding of TCDD to cytosolic AHR and no effect on the DMBA- or TCDD-activated binding of the AHR to the CYP1A1 promoter (data not shown). These data indicate that DHEA does not carry out its inhibitory activity with regard to CYP1A1 expression by affecting the ligand-induced transcription of CYP1A1.

Although there are numerous studies that demonstrate that CYP1A1 expression is controlled primarily at the transcriptional level, one study indicated that post-transcriptional mechanisms, i.e. mRNA stability, may play a role in determining the level of CYP1A1 mRNA (35). Because our data indicate that DHEA decreases CYP1A1 mRNA but does not affect CYP1A1 transcription, we examined the effect of DHEA on CYP1A1 mRNA degradation by carrying out actinomycin D chase experiments. These experiments indicate that DHEA significantly shortens the half-life of CYP1A1 mRNA (Figs. 7 and 8). This increased mRNA degradation seems to be selective because GAPDH mRNA was unaffected by DHEA. The inhibition of CYP1A1 expression by DHEA therefore appears to occur at a post-transcriptional level through modulation of CYP1A1 mRNA stability. Although mRNA stability plays a major role in the determination of gene expression, the regulation of mRNA stability is poorly understood, and the mechanism by which DHEA affects mRNA stability remains to be studied. The modulation of CYP1A1 mRNA stability by a chemopreventive compound is novel and may represent an important mechanism of chemoprevention.

The current data are the first demonstration, to our knowledge, that DHEA inhibits the expression of CYP1A1. This provides a possible explanation for the potent chemopreventive activity of DHEA with regard to the initiation of chemically induced carcinogenesis. In this model system, this inhibition occurs at physiologically relevant concentrations. Thus, DHEA may serve to modulate the response to xenobiotics in vivo. This may be a heretofore unrecognized physiologic function of DHEA.

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