Biochemical Pharmacology, Vol. 37, No. 6, pp. 1003–1008, 1988.
Printed in Great Britain.

0006-2952/88 $3.00 + 0.00
Pergamon Press plc

EFFECT OF 17α-ETHYNYLESTRADIOL ON THE INDUCTION OF CYTOCHROME P-450 BY 3-METHYLCHOLANTHRENE IN CULTURED CHICK EMBRYO HEPATOCYTES

SUSAN A. SUNDSTROM,* JACQUELINE F. SINCLAIR,†‡§ E. LUCILE SMITH†‡ and PETER R. SINCLAIR†‡

Departments of * Pharmacology and Toxicology and † Biochemistry, Dartmouth Medical School, Hanover, NH 03756; and ‡ VA Medical Center, White River Junction, VT 05001, U.S.A.

(*Received* 9 *February* 1987; *accepted* 27 *July* 1987)

Abstract—This study investigated the effects of estrogens on the induction of cytochrome P-450 by polycyclic aromatic hydrocarbons in primary cultures of chick embryo hepatocytes. Exposure to polycyclic aromatic hydrocarbons, such as 3-methylcholanthrene led to 2- to 3-fold increases of cytochrome P-450. The amount of cytochrome P-450 induced by 3-methylcholanthrene was increased 40–50% when the synthetic estrogen, 17α-ethynylestradiol, was also present. The rate of decay of cytochrome P-450 in the presence of cycloheximide as measured spectrophotometrically was similar in cells previously treated with either 3-methylcholanthrene or 3-methylcholanthrene plus 17α-ethynylestradiol, suggesting that 17α-ethynylestradiol did not affect the stability of the 3-methylcholanthrene-induced cytochrome P-450. In contrast, 17α-ethynylestradiol did not potentiate the induction of cytochrome P-450 by phenobarbital-like inducers, such as 2-propyl-2-isopropylacetamide, as indicated by a lack of increase in both the content of cytochrome P-450 and benzphetamine demethylase activity. The naturally occurring estrogens, 17β-estradiol and estrone, and the synthetic estrogen, diethylstilbestrol, did not affect cytochrome P-450 induction by 3-methylcholanthrene, suggesting that the effect of 17α-ethynylestradiol was not mediated via the estrogen receptor. We investigated whether the amount of cytochrome P-450 increased in the presence of 17α-ethynylestradiol was the same or different from that induced by 3-methylcholanthrene. Treatment with 17α-ethynylestradiol alone resulted in a small increase in ethoxyresorufin deethylase activity. The enzymatic activities of 7-ethoxyresorufin and aryl hydrocarbon hydroxylase, when expressed per cytochrome P-450 content, were identical in microsomes from cells treated with either 3-methylcholanthrene or the combination of 3-methylcholanthrene and 17α-ethynylestradiol. The data suggest that the additional cytochrome P-450 induced by the combination of 17α-ethynylestradiol and 3-methylcholanthrene was the same isozyme as that induced by 3-methylcholanthrene alone.

The cytochromes P-450 are a family of isozymes involved in oxidative metabolism of a number of exogenous and endogenous substrates such as drugs, pesticides, steroid hormones and fatty acids. Different isozymes of the enzyme can be induced by treatment of animals with specific compounds [1]. There have been at least twelve isozymes purified from rat liver and several from rabbits and mice [1, 2]. Polycyclic aromatic hydrocarbons, such as 3-methylcholanthrene, induce at least two cytochrome P-450s designated cytochrome P-450c and cytochrome P-450d in the rat [3, 4]. In chick embryo hepatocytes, MC‖ induces a major isozyme of 60,000 molecular weight, whereas glutethimide, a PB-like inducer of cytochrome P-450, induces a major isozyme of 50,000 molecular weight [5, 6]. An additional isozyme of 55,000 molecular weight has also been isolated from the liver of PB-treated roosters [6, 7]. Currently, it is not known whether more than one isozyme is induced by MC in chicken embryos.

In the present study, we examined the effects of a synthetic estrogen, EE2, on the induction of cytochrome P-450 in chick embryo liver cell cultures. EE2 is a risk factor in two clinical disorders thought to involve the MC-inducible isozyme of cytochrome P-450: hepatocarcinogenesis [8] and porphyria cutanea tarda [9]. Many people are exposed to both EE2 therapeutically as well as to polycyclic aromatic hydrocarbons which are ubiquitous in the environment. Therefore, we investigated the effects of the combination of EE2 and polycyclic hydrocarbons on the induction of cytochrome P-450.

Cultured chick embryo hepatocytes were used as the system of investigation because they retain PB- and MC-mediated induction of cytochrome P-450 to levels induced in the intact embryo and adult chicken

§ All correspondence should be sent to: Jacqueline F. Sinclair, Research Service (151-A), Veterans Administration, White River Junction, VT 05001.

‖ Abbreviations: MC, 3-methylcholanthrene; PB, phenobarbital; TCB, 3,4,3′,4′-tetrachlorobiphenyl; PIA, 2-propyl-2-isopropylacetamide; βNF, β-naphthoflavone; TCDD, 2,3,7,8-tetrachloro-*p*-dibenzodioxin; PAH, polycyclic aromatic hydrocarbon; EE2, 17α-ethynylestradiol; E2, 17β-estradiol; E1, estrone; DES, diethylstilbesterol; EROD, 7-ethoxyresorufin-*O*-deethylase; BPHET, benzphetamine demethylase; AHH, aryl hydrocarbon hydroxylase; DEX, dexamethasone; T3, 3,3′,5-triiodothyronine; and HEPES, *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid.

[10–12]. The patterns of cytochrome P-450 induction are similar to those in the intact chicken embryo, adult chicken and adult rodents [10–12]. Also, tissue culture affords the major advantages of being a defined environment and restricting effects of the hormones to the liver, without interference from the hypothalamus, sex organs or adrenal glands.

This study shows that 17α-ethynylestradiol potentiated cytochrome P-450 induction by MC-like inducers in chick embryo liver cultures. The enzymatic analyses suggest that the particular isozyme induced by MC and EE2 is the same as that induced by MC alone.

METHODS

Preparation and treatment of cultured chick embryo hepatocytes. Primary cultures of livers from 16 to 17-day chicken embryos were prepared as described previously [13]. The hepatocytes were plated in Williams E medium containing per ml: 1.0 μg insulin, 0.3 μg dexamethasone (DEX) and 1.0 μg 3,3′,5-triiodothyronine (T3). Twenty hours after plating the cells, the medium was changed to Williams E containing 20 mM HEPES buffer, pH 7.4, DEX and T3, and chemicals were added in either dimethyl sulfoxide or ethanol (not more than 2 μl/ml of medium) as indicated in the figure legends. Solvents without chemicals were added to control plates.

Preparation of microsomes from cell cultures. Cultures (10-cm plates) were rinsed with 20 mM Tris–HCl–0.25 M sucrose, pH 7.4, at room temperature and harvested in 1.0 ml/plate of the same buffer at 4°. The cell suspensions from ten 10-cm plates were combined and homogenized with a Potter–Elvehjem homogenizer (600 rpm, 15 strokes). The homogenate was sonicated at a setting of 2 for 13 sec, using a model W140D Sonifer Cell disrupter (Heat Systems Ultrasonics, Inc.). The sonicate was centrifuged at 10,000 *g* for 10 min, and the supernatant fraction was centrifuged at 105,000 *g* for 1 hr. The microsomal pellet was overlayed with 0.1 M sodium phosphate, pH 7.4, and frozen at −20° for no more than 1 week and then resuspended in the same buffer. We found that the cytochrome P-450 activity is stable to freezing over this time period.

Assays. Cytochrome P-450 was measured spectrophotometrically either in the 8700 *g* supernatant fraction from detergent solubilized cell homogenates or in the microsomal fraction as described previously [11]. In different experiments, the levels of cytochrome P-450 in untreated cells varied from 40 to 60 pmol/mg protein; however, the extent of increase in cytochrome P-450 after exposure to inducers was similar. Benzphetamine demethylase activity was measured in cell homogenates as previously described [13]. Aryl hydrocarbon (benzo[*a*]pyrene) hydroxylase activity was measured in microsomes by a direct fluorometric method of Yang and Kicha [14], which measures the rate of decrease in benzo[*a*]pyrene concentration. We found that the assay was linear from 0.05 to 0.25 mg native microsomal protein per assay in the presence of 0.2 mg denatured microsomal protein (obtained from untreated chick embryo liver) per assay. The fluorescence change was recorded with a Perkin–Elmer 512 spectrofluorometer with the following settings: excitation, 387 nm; emission, 405 nm; excitation slit width, 10 nm; emission slit width, 3 nm. 7-Ethoxyresorufin-*O*-deethylase activity was measured by a direct fluorometric technique using the following modifications of an assay published previously by Burke *et al.* [15]: aliquots of resuspended microsomes were added to 2 ml buffer (0.1 M sodium phosphate, pH 7.8, containing 200 μg/ml bovine serum albumin) at 25°. 7-Ethoxyresorufin (3 nmol) was added, and the baseline was recorded. The reaction was initiated with 50 μl of NADPH (1 mM). The rate of resorufin production was recorded on the spectrofluorometer with the following settings: excitation, 530 nm; emission, 590 nm; slits, 20 nm. The amount of resorufin was determined using a known standard. Protein was determined by the method of Lowry *et al.* [16], using bovine serum albumin as a standard.

Sources of chemicals. Williams E medium and trypsin (TC59) were purchased from Gibco Laboratories (Grand Island, NY). βNF was purchased from the Aldrich Chemical Co. (Milwaukee, WI); 3MC (puriss. grade) was from Fluka (Hauppauge, NY); and TCB was from Ultra Scientific (Hope, RI). PIA was a gift from Hoffmann-LaRoche (Nutley, NJ); ethanol (USP grade) was supplied by the U.S. Industrial Chemical Co. (Tuscola, IL); HEPES buffer was obtained from the Calbiochem-Behring Corp. (La Jolla, CA); and Emulgen 913 was a gift from the Kao Soap Co. (Tokyo, Japan). Bovine serum albumin, insulin, T3, NADPH, $NADP^+$, nicotinamide, DL-isocitrate, isocitrate dehydrogenase, estrone and benzo[*a*]pyrene were all purchased from the Sigma Chemical Co. (St. Louis, MO). Dexamethasone (sodium phosphate salt) was from the Lemmon Co. (Sellersville, PA). 7-Ethoxyresorufin was purchased from Pierce Chemicals (Rockford, IL); resorufin was obtained from Pfaltz & Bauer (Stamford, CT); and benzphetamine was a gift from the Upjohn Co. (Kalamazoo, MI). Repurified 17β-estradiol and 17α-ethynylestradiol were supplied by Dr. W. Slikker (National Center for Toxicological Research, Jefferson, AK). Diethylstilbestrol was obtained from the U.S. Biochemical Corp. (Cleveland, OH).

Statistics. Data are expressed as the mean of triplicate plates ± the standard deviation unless indicated otherwise in the figure legends.

RESULTS

Effect of estrogens on induction of cytochrome P-450. Figure 1 shows the effects of EE2 and MC-like inducers (MC, TCB, and βNF) on cytochrome P-450 levels measured spectrophotometrically. EE2 alone did not increase the amount of cytochrome P-450 compared with control cells. βNF, TCB and MC induced cytochrome P-450 2- to 3-fold over control cells at doses and times (20–24 hr) previously shown to maximally induce cytochrome P-450 in these cells ([17]; and P. R. Sinclair, W. J. Bement and J. F. Sinclair, unpublished observations). The combination of EE2 and the MC-like inducers increased cytochrome P-450 at least 40% over that produced by the MC-like inducer alone. With all chemical

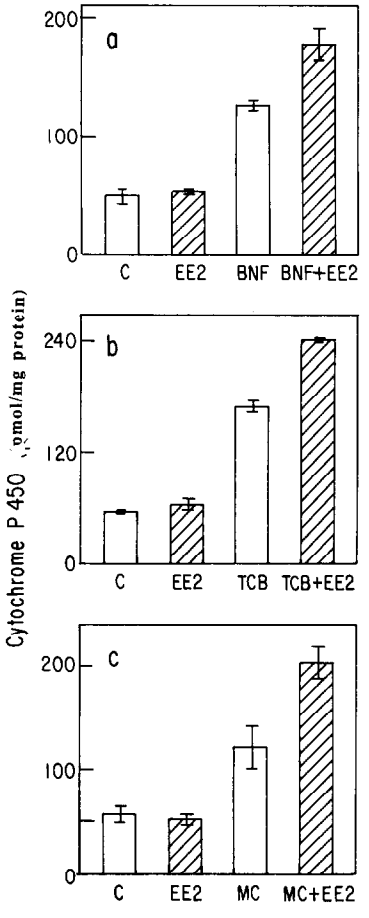

Fig. 1. Induction of cytochrome P-450 by MC, TCB, or βNF in the presence of EE2. Cells were treated with EE2 (15 μM) alone; βNF (15 μM) (a); TCB (68 nM) (b); or MC (0.93 μM) (c); or with EE2 and the MC-like inducer of cytochrome P-450 for 24 hr. Cytochrome P-450 was determined in the 8700 *g* supernatant fraction of cell homogenates as described in Methods. Hatched bars represent the presence of estrogens in this and subsequent figures.

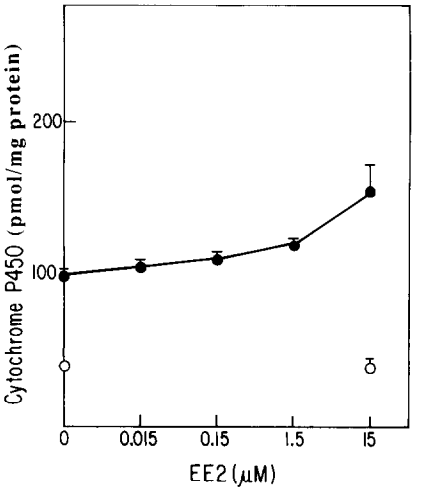

Fig. 2. Dose–response of EE2 on MC-mediated cytochrome P-450 induction. Cells were treated with EE2 (○) or EE2 and MC (●) (0.93 μM) for 24 hr. Cytochrome P-450 was determined in the 8700 *g* supernatant fraction of cell homogenates.

inducers of cytochrome P-450, maximal induction was observed after 20–24 hr exposure (results not shown). In dose–response studies, we found that 1.5 μM EE2 caused an increase in cytochrome P-450 over MC alone; however, 15 μM EE2 was required for the maximal synergistic increase in cytochrome P-450 (Fig. 2).

PIA and ethanol have been shown to increase a phenobarbital-induced isozyme(s) of cytochrome P-450 in this system [4, 10, 13]. EE2 caused no further increase in cytochrome P-450 over that induced by either PIA or ethanol (results not shown).

The naturally occurring estrogens, E2 and E1, as well as the synthetic, non-steroidal estrogen, DES, were also tested for possible effects on cytochrome P-450 induction. DES (7.5 and 15 μM), E1 and E2 (15 μM) all did not potentiate induction of cytochrome P-450 by MC (results not shown).

Enzymatic characterization of the cytochrome P-450 induced by EE2 and MC-like inducers. In cultured chick embryo hepatocytes, benzphetamine demethylase activity is increased in cells treated with phenobarbital-like inducers such as PIA [13]. 7-Ethoxyresorufin-*O*-deethylase activity has been shown to be specific for the MC-inducible isozyme of cytochrome P-450 in rats [4] and in MC-treated cultured chick embryo hepatocytes [13]. Aryl hydrocarbon hydroxylase activity has also been used to characterize cytochrome P-450 activity induced by MC [14]. We measured all three catalytic activities in cells treated with the combination of EE2 and different inducers of cytochrome P-450.

In Fig. 3, AHH activities in control microsomes and microsomes from cells treated with EE2, MC,

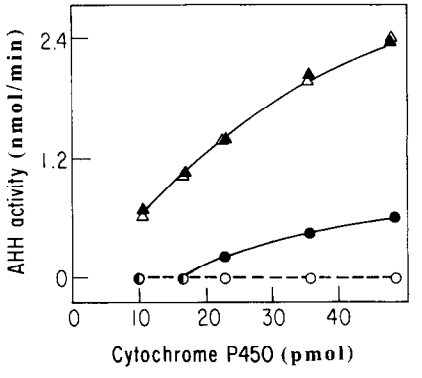

Fig. 3. Effect of EE2 on induction of AHH activity. Cells were treated with EE2 (15 μM), MC (0.93 μM), or EE2 and MC for 24 hr. Cells from ten 10-cm plates per treatment were harvested together, and microsomes were prepared as described in Methods. AHH activity was measured fluorometrically in samples containing increasing amounts of microsomal cytochrome P-450. Each measurement was made in triplicate. (Unless indicated otherwise, standard deviations lie within the symbol.) Cytochrome P-450 concentrations were as follows (pmol/mg protein): control (69), (○); EE2 (79) (●); MC (270) (△); and MC + EE2 (484) (▲).

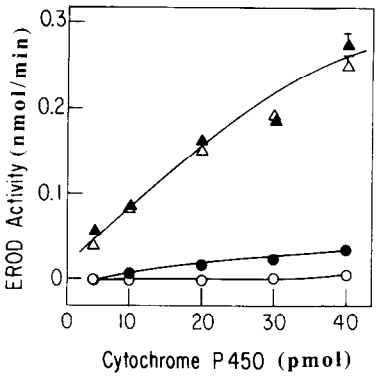

Fig. 4. Effect of EE2 on induction of EROD activity. Cells were treated with EE2 (15 μM), MC (0.93 μM), or MC and EE2 for 24 hr. EROD activity was measured fluorometrically in samples containing increasing amounts of microsomal cytochrome P-450. Each measurement was made in duplicate. Cytochrome P-450 concentrations were as follows (pmol/mg protein): control (93) (○); EE2 (100) (●); MC (280) (△); and MC + EE2 (501) (▲).

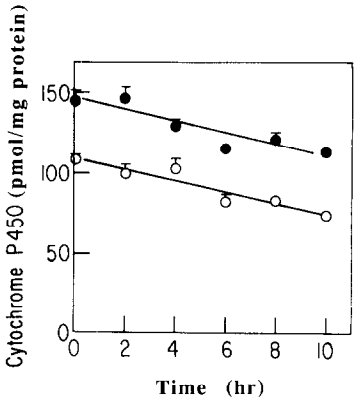

Fig. 6. Decay of MC-induced cytochrome P-450 in the presence and absence of EE2. Cells were treated with MC (○) (0.93 μM) or MC and EE2 (●) (15 μM) for 21 hr, and cycloheximide (1.78 μM, in water) was then added to each plate. Cytochrome P-450 concentrations were determined at various times after cycloheximide treatment. The lines were generated by linear regression analysis with correlation coefficients for the MC and MC + EE2 conditions of 0.95 and 0.92 respectively.

or MC combined with EE2 are plotted as a function of cytochrome P-450 concentration. Microsomes from control cells did not have measurable AHH activity. Cells treated with EE2 had a small amount of microsomal AHH activity at cytochrome P-450 concentrations above 20 pmol/reaction. AHH activity was increased in microsomes from cells treated with MC both in the presence and absence of EE2, and the activity increased with increasing amounts of cytochrome P-450. On the basis of cytochrome P-450 content, microsomes from cells treated with EE2 and MC versus MC alone had identical AHH activity.

EROD activity was also measured in microsomes as a function of cytochrome P-450 concentration (Fig. 4). EROD activity was barely detectable in control cells. In microsomes from cells treated with EE2 alone, there was a slight increase in EROD activity with increasing amounts of cytochrome P-450. As with AHH, cells treated with EE2 and MC versus MC alone had identical EROD activities on the basis of cytochrome P-450 content.

Treatment with EE2 had no effect on BPHET activity in either control cells or cells treated with MC- or PB-like inducers of cytochrome P-450 (Fig. 5).

Effect of EE2 on cytochrome P-450 degradation. We investigated whether EE2 increased cytochrome P-450 by merely preventing cytochrome P-450 degradation by measuring the decrease of cytochrome P-450 with time following the addition of cycloheximide (Fig. 6). The dose of cycloheximide used (0.5 μg/ml medium) has been shown previously to cause 85% inhibition of protein synthesis in these cells [18]. In the absence of cycloheximide, cytochrome P-450 levels in MC-treated cells are maintained over 48 hr [13]. In the presence of cycloheximide, the concentration of cytochrome P-450 as measured spectrophotometrically decreased linearly with time and at the same rate whether or not EE2 was present (Fig. 6).

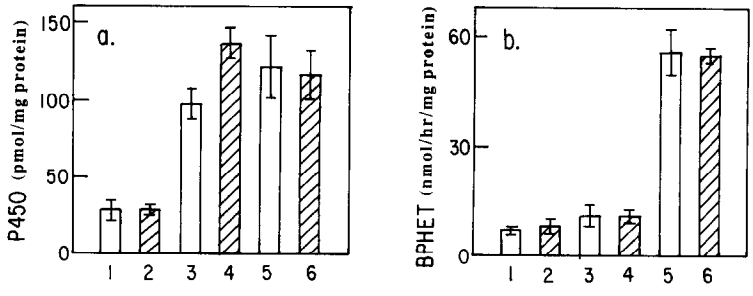

Fig. 5. Effect of EE2 on induction of cytochrome P-450 and on BPHET activity. Cells were treated with EE2 (15 μM), MC (0.93 μM), PIA (140 μM), or EE2 plus MC or PIA for 24 hr. BPHET activity was measured in whole homogenates, and cytochrome P-450 was determined in the 8700 *g* supernatant fraction of cell homogenates. Key: (1) control; (2) EE2; (3) MC; (4) MC + EE2; (5) PIA; and (6) PIA + EE2.

DISCUSSION

The results of this study show that EE2 potentiated cytochrome P-450 induction by polycyclic aromatic hydrocarbons in chick embryo liver cells. Enzymatic characterization suggested that the cytochrome P-450 induced by the combination of MC-like compounds and EE2 was the same isozyme as that induced by MC alone, since the increases in EROD and AHH were directly proportional to the increases in cytochrome P-450 as measured spectrophotometrically (Figs. 3 and 4). In rats, MC-like inducers of cytochrome P-450 increase at least two immunochemically related cytochrome P-450 isozymes termed cytochrome P-450c and cytochrome P-450d [3, 4] which differ greatly in their abilities to catalyse AHH [3] and EROD [19]. Antibodies to the 60,000 molecular weight protein induced by MC in chick embryos inhibit almost all EROD activity [5] which suggests that only one isozyme catalyzes EROD activity. However, it is still possible that MC induces more than one isozyme in cultured chick embryo hepatocytes.

One possible mechanism for the EE2 enhancement of cytochrome P-450 induction is that EE2 stabilizes the MC-inducible cytochrome P-450 against breakdown possibly by binding to the cytochrome P-450. Steward and co-workers [20] showed that, in cultured hepatocytes from isosafrole-treated rats, cytochrome P-450d is stabilized by the addition of isosafrole to the cells, most likely due to an association of isosafrole with the cytochrome. However, our results suggest that EE2 did not stabilize the MC-inducible protein against degradation, since the rates of degradation of cytochrome P-450 in the presence of cycloheximide were similar for both the MC-induced isozyme and that induced by MC plus EE2 (Fig. 6). If EE2 or a metabolite had been bound to the cytochrome P-450, it was not bound tightly to the active site, since the microsomal cytochrome P-450 was enzymatically active (Figs. 3 and 4). If loosely bound, the complex must have been dissociated during preparation of microsomes or addition of substrate.

Several studies have shown that EE2 and other compounds containing vinyl and ethynyl groups cause destruction of cytochrome P-450 [21]. EE2 incubated with rat hepatocytes or microsomes and NADPH or administered acutely to rats *in vivo* causes a rapid loss (in 1 hr) of cytochrome P-450 [22, 23]. In contrast, we found no decrease in cytochrome P-450 in control and PAH-treated cells exposed to 15 μM EE2 at either 24 hr (Figs. 1 and 2), 30 min or 4 hr after the addition of EE2 (results not shown). In the studies reported in this manuscript, cytochrome P-450 was measured at 24 hr after exposure to EE2. Since EE2 was almost completely conjugated by 4 hr, by 24 hr little EE2 would have remained to cause any destruction of cytochrome P-450 (S. A. Sundstrom, Z. Althaus, W. Slikker, Jr. and J. F. Sinclair, unpublished observations).

Recently, it has been reported that dexamethasone acts synergistically with polycyclic aromatic hydrocarbons to increase the MC-inducible cytochrome P-450 in cultures of fetal hepatocytes from humans or rats [24, 25]. Potentiation of the MC induction of cytochrome P-450 was suggested to be mediated via the glucocorticoid receptor, possibly by an interaction between the receptor–glucocorticoid complex and the cytochrome P-450c gene [25]. In intact rats, a separate isozyme of cytochrome P-450 induced by glucocorticoids has been identified in the liver that appears to be distinct from the cytochrome P-450 induced by polycyclic aromatic hydrocarbons [26]. This cytochrome P-450 is designated cytochrome P-450p, and its induction may not be mediated via the glucocorticoid receptor [27]. In cultured chick hepatocytes, the potentiation of the MC induction of cytochrome P-450 by EE2 was not mediated by binding to the glucocorticoid receptor since the synthetic glucocorticoid dexamethasone was present in all the media during exposure to inducers at concentrations (0.76 μM) most likely to saturate the receptor [28]. In addition, in contrast to EE2, DEX did not, by itself, increase EROD activity since no EROD activity was detectable in cells exposed to DEX alone (Fig. 4). In addition, DEX did not potentiate the MC-mediated induction of P-450 since both the content of cytochrome P-450 and EROD activity were the same in MC-treated cells whether or not DEX was included in the medium (results not shown).

MC-mediated induction of cytochrome P-450 was not potentiated by the estrogens E2, E1 or DES (results not shown), indicating that the response was specific for EE2. Furthermore, the potentiation of the MC-mediated induction of cytochrome P-450 by EE2 is unlikely to be mediated by the estrogen receptor since DES, a synthetic estrogen with greater affinity for the estrogen receptor than EE2 [29–31], did not increase the MC-mediated induction of cytochrome P-450.

Induction of cytochrome P-450 by MC is mediated via binding to a cytosolic protein termed the Ah receptor [32]. The receptor complex is translocated to the nucleus where it may activate particular genes leading to increases in transcription [32]. EE2 may increase binding of MC to the receptor. However, this would not explain the further increase in cytochrome P-450 since compounds with greater affinity for the Ah receptor than MC, such as TCDD, do not increase the maximal amount of cytochrome P-450 induced, but merely cause maximal induction at a lower dose [33]. Since EE2 increased the maximal amount of cytochrome P-450 induced by MC (Figs. 1 and 2), the evidence suggests that EE2 does not increase the binding of MC to the Ah receptor. Alternatively, EE2 treatment may increase the amount of Ah receptor.

Another possibility may involve EE2-mediated increases in transcription or stability of mRNA independent of the Ah receptor. There is evidence in mice that induction of cytochrome P-450 by PAH is regulated by a labile repressor protein that inhibits transcription of the cytochrome P1-450 gene [34]. If this repressor protein also exists in chick embryo hepatocytes, a decrease of such a repressor protein by EE2 is another possible mechanism for the observed increase in cytochrome P-450.

The potentiation of the MC-inducible cytochrome P-450 by EE2 may be an important consideration in explaining the mechanism by which oral con-

traceptives are involved in carcinogenesis [8, 9, 35]. Various synthetic steroids have been classified as carcinogens. However, studies in rats showed that these compounds were unable to initiate tumor activity by themselves [36]. The MC-inducible isozyme of cytochrome P-450 is involved in the metabolism of numerous polycyclic aromatic hydrocarbons associated with mutagenesis and carcinogenesis [37]. Metzler [8] has suggested that metabolism of estrogens to reactive intermediates by cytochrome P-450 may be important in the mechanism of estrogen-related carcinogenicity. In the presence of βNF, EE2 induces hepatic tumors in hamsters [38]. In addition, contraceptive steroids have been implicated as cocarcinogens for dimethylbenzanthracene-induced mammary tumors in the hamster [39]. Helton and Goldzieher [40] proposed that the relationship between synthetic estrogens and chemical carcinogens might be mediated through liver monooxygenases. Our results are consistent with this hypothesis and suggest that EE2 may enhance the activation of carcinogens by increasing PAH-mediated induction of cytochrome P-450.

The synthetic estrogen EE2 has been implicated as a risk factor in the development of porphyria cutanea tarda in humans [9]. Porphyria cutanea tarda is characterized by hepatic accumulation and urinary excretion of uroporphyrin [9]. A number of halogenated aromatic compounds cause accumulation of uroporphyrin in both intact animals [41] and cultured chick embryo hepatocytes [42]. In both systems, an MC-inducible form of cytochrome P-450 may have a role in the development of uroporphyria [17, 43] and the amount of the cytochrome P-450 may be rate-limiting for uroporphyrin accumulation [17]. EE2 may therefore increase the risk of porphyria cutanea tarda by increasing the amount of the MC-inducible cytochrome P-450.

In summary, we have shown that the induction of cytochrome P-450 by MC-like compounds was potentiated by the synthetic estrogen, EE2. Enzymatic studies suggest that the increased cytochrome P-450 was the same isozyme as that induced by MC-like chemicals alone.

Acknowledgements—This work was supported by the VA, NIH (CA25012) and ES 07104. We wish to thank Dr. Joshua Hamilton for his assistance with the AHH assay, Dr. Joyce Goldstein for helpful discussions, and Mr. Ronald Chapman for typing the manuscript.

REFERENCES

1. A. Y. H. Lu and S. B. West, *Pharmac. Rev.* **31**, 277 (1980).
2. D. J. Waxman, *Cytochrome P-450: Structure, Mechanism and Biochemistry* (Ed. P. R. Ortiz de Montellano), p. 525. Plenum, New York (1986).
3. D. Ryan, P. Thomas and W. Levin, *J. biol. Chem.* **255**, 7941 (1980).
4. P. E. Thomas, L. M. Reik, D. E. Ryan and W. Levin, *J. biol. Chem.* **258**, 4590 (1983).
5. P. Sinclair, J. Frezza, J. Sinclair, J. Bement, J. Healey and H. Bonkovsky, *Fedn Proc.* **45**, 1663 (1986).
6. L. Oron and S. Bar-Nun, *Biochem. biophys. Res. Commun.* **125**, 1096 (1984).
7. I. Ades, *IRCS med. Sci.* **11**, 302 (1983).
8. M. Metzler, *Archs Toxic.* **55**, 104 (1984).
9. G. D. Sweeney, *Clin. Biochem.* **19**, 3 (1986).
10. F. R. Althaus, J. F. Sinclair, P. Sinclair and U. Meyer, *J. biol. Chem.* **254**, 2148 (1979).
11. J. F. Sinclair, P. R. Sinclair and H. L. Bonkowsky, *Biochem. biophys. Res. Commun.* **86**, 710 (1979).
12. S. J. Buynitsky, A. E. Wade, J. F. Munnell and W. L. Ragland, *Drug Metab. Dispos.* **6**, 1 (1978).
13. J. F. Sinclair, P. R. Sinclair, E. L. Smith, W. J. Bement, J. Pomeroy and H. Bonkowsky, *Biochem. Pharmac.* **30**, 2805 (1981).
14. C. S. Yang and L. P. Kicha, *Analyt. Biochem.* **84**, 154 (1978).
15. M. D. Burke, S. Thompson, C. R. Elcombe, J. Halpert, T. Haaparanta and R. T. Mayer, *Biochem. Pharmac.* **34**, 3337 (1985).
16. O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. biol. Chem.* **193**, 265 (1951).
17. P. R. Sinclair, W. J. Bement, H. L. Bonkovsky and J. F. Sinclair, *Biochem. J.* **222**, 737 (1984).
18. J. F. Sinclair, L. Smith, W. J. Bement, P. R. Sinclair and H. L. Bonkowsky, *Biochem. Pharmac.* **31**, 2811 (1982).
19. A. Astrom and J. De Pierre, *Carcinogenesis* **6**, 113 (1985).
20. A. R. Steward, S. A. Wrighton, D. S. Pasco, J. B. Fagan, D. Li and P. S. Guzelian, *Archs Biochem. Biophys.* **241**, 494 (1985).
21. P. R. Ortiz de Montellano and M. A. Correia, *A. Rev. Pharmac. Toxic.* **23**, 481 (1983).
22. I. N. H. White and U. Muller-Eberhard, *Biochem. J.* **166**, 57 (1977).
23. D. C. Blakey and I. N. H. White, *Biochem. Pharmac.* **35**, 1561 (1986).
24. J. M. Mathis, R. A. Prough, R. N. Hines, E. Bresnick and E. R. Simpson, *Archs Biochem. Biophys.* **246**, 439 (1986).
25. J. M. Mathis, R. A. Prough and E. R. Simpson, *Archs Biochem. Biophys.* **244**, 650 (1986).
26. N. A. Elshourbagy and P. S. Guzelian, *J. biol. Chem.* **255**, 1279 (1980).
27. E. G. Schuetz and P. S. Guzelian, *J. biol. Chem.* **259**, 2007 (1984).
28. J. D. Baxter and G. M. Tomkins, *Proc. natn. Acad. Sci. U.S.A.* **68**, 932 (1971).
29. W. B. Dandliker, R. J. Brawn, M. L. Hsu, P. N. Brawn, J. Levin, C. Y. Meyers and V. M. Kolb, *Cancer Res.* **38**, 4212 (1978).
30. K. Fotherby, *Meth. Find. exptl Clin. Pharmac.* **4**, 133 (1982).
31. R. B. Dickson and A. J. Eisenfeld, *Endocrinology* **108**, 1511 (1981).
32. A. Poland, E. Glover and A. S. Kende, *J. biol. Chem.* **251**, 4936 (1976).
33. A. Poland and J. C. Knutson, *A. Rev. Pharmac. Toxic.* **22**, 517 (1982).
34. J. P. Whitlock Jr., *A. Rev. Pharmac. Toxic.* **26**, 333 (1986).
35. R. H. Purdy and M. V. Marshall, *Carcinogenesis* **5**, 1709 (1984).
36. H. Ochs, B. Dusterberg, P. Gunzel and R. Schulte-Hermann, *Cancer Res.* **46**, 1224 (1986).
37. A. H. Conney, *Cancer Res.* **42**, 4875 (1982).
38. J. J. Li and S. A. Li, *Archs Toxic.* **55**, 110 (1984).
39. P. J. Hertzog, V. Ilacqua, M. Rustia and R. Gingell, *Toxic. appl. Pharmac.* **54**, 340 (1980).
40. E. D. Helton and J. W. Goldzieher, *J. Toxic. environ. Hlth.* **3**, 231 (1977).
41. G. H. Elder, in *Handbook of Experimental Pharmacology* (Eds. F. De Matteis and W. N. Aldridge), Vol. 44, p. 157. Springer, New York (1978).
42. P. R. Sinclair and S. Granick, *Biochem. biophys. Res. Commun.* **61**, 124 (1974).
43. K. G. Jones, F. M. Cole and G. D. Sweeney, *Toxic. appl. Pharmac.* **61**, 74 (1981).