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INDUCTION *IN VITRO* AND COMPLETE CODING REGION SEQUENCE OF CYTOCHROME P4501A1 cDNA FROM CULTURED WHOLE RAT CONCEPTUSES DURING EARLY ORGANOGENESIS

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Abstract—Exposures of cultured whole rat conceptuses during organogenesis to 3-methylcholanthrene (MC; 0.025–25 μM), 5,6-benzoflavone (BNF; 5–100 μM) or benz[*a*]anthracene (BA; 5–100 μM) were effected by placement of each of these "MC-type" inducing agents in the culture medium at the time of explantation on day 9.5 of gestation. Conceptuses were then cultured for 48 hr and evaluated on day 11.5 for increased expression of inducible conceptal cytochrome P450 (P450). The three agents each elicited concentration-dependent increases in 7,8-benzoflavone (ANF)-inhibitable ethoxyresorufin *O*-deethylase (EROD) activities and increased P4501A1 mRNA as detected by primer-specific reverse transcriptase-polymerase chain reaction (RT-PCR) in cell-free preparations of the treated, cultured conceptuses. At effective inducing concentrations, dysmorphogenic or other embryotoxic effects were not detectable. At 20 μM concentrations, the three agents exhibited roughly equal induction that was approximately equivalent in magnitude (6- to 13-fold) to that achieved previously with exposures to MC *in utero*. Additions to the culture medium of 2.5 to 10 μM concentrations of dexamethasone (DEX) did not alter significantly the magnitude of MC-elicited induction *in vitro*. Repeated full-length sequencing of an RT-PCR-amplified cDNA revealed a coding region sequence identical to that reported for the P4501A1 sequence from adult rat liver. The results provide a basis for investigations, in the absence of maternal influences, of the regulation of mammalian conceptal P4501A1 in intact tissues during organogenesis, a gestational period critical in terms of the dysmorphogenic and other embryotoxic effects of foreign organic chemicals. The results are also pertinent to studies of embryotoxicity, particularly to the transplacental carcinogenicity, mutagenicity and dysmorphogenicity of P4501A1 substrates.

Key words: P4501A1; cytochrome P450; embryogenesis; sequence analysis; teratogenesis; enzyme induction

P450‡ plays a central role in the biotransformation of xenobiotics, such as drugs and other environmental chemicals, and of liposoluble endobiotics, such as steroid hormones, bilirubin, eicosanoids and fatty acid derivatives [1–4]. Studies from this laboratory have demonstrated that expression of even very low levels of P450 isoforms in conceptal tissues can play a dramatic role in the capacity of certain environmental chemicals to elicit dysmorphogenic and other embryotoxic effects in developing embryos [reviewed in Refs. 5–7]. Prior exposure of conceptuses *in utero* to MC resulted in a marked increase in the capacity of AAF (a P4501A substrate) to produce dysmorphogenic effects in the same conceptuses in culture. This AAF-elicited dysmorphogenesis was selectively blocked by carbon monoxide, apparently via inhibition of conceptal P450-catalyzed bioactivation of AAF to reactive, dysmorphogenic, intermediary metabolites of which catechols and quinoneimines appeared to be of the greatest importance. Other studies [8–10] strongly suggested that MC, a potent inducer of P4501A isoforms, elicits induction *in utero* of P4501A1 (nomenclature from Ref. 3) or a closely related isoform(s) in several tissues of the rat conceptus between days 10 and 14 of gestation.

Of pertinence to these issues, however, are the recent reports [11, 12] that a P450 isoform clearly distinct from P4501A1/2 is inducible by TCDD in 10T1/2 mouse embryo fibroblasts. TCDD is likewise a potent "MC-type" inducer of P4501A isoforms and the isoform induced in mouse embryo fibroblasts (termed P450EF by the authors) actively catalyzed the biotransformation of polynuclear aromatic hydrocarbons, classical substrates of P4501A1. The same or a closely related isoform was reported more

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‡ Abbreviations: MC, 3-methylcholanthrene; BNF, 5,6-benzoflavone; BA, benz[*a*]anthracene; EROD, 7-ethoxyresorufin *O*-deethylase; MROD, 7-methoxyresorufin *O*-demethylase; PROD, 7-pentoxyresorufin *O*-depentylase; BZROD, 7-benzyloxyresorufin *O*-debenzylase; RT-PCR, reverse transcriptase-polymerase chain reaction; DEX, dexamethasone; AAF, 2-acetylaminofluorene; TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; ANF, 7,8-benzoflavone; P450, cytochrome P450 protein; *CYP*, cytochrome P450 gene or cDNA; and Ah, aryl hydrocarbon.

recently to be induced by both TCDD and BA in stromal cells from mouse uterine endometrium [13]. More recently, Weaver *et al.* [14] reported the detection of a possible variant isoform of rat P4501A1 (termed P450MCX). This isoform also exhibited induction by MC but displayed a substrate specificity differing somewhat from that of P4501A1. These investigations have raised the question as to whether an MC-inducible P450 isoform detected by us in rat conceptuses during organogenesis was in fact P4501A1 or perhaps a closely related but sequentially separate isoform(s) with similar enzymic and regulatory properties. The question of conceptal P450 isoform identity is further reinforced by other considerations, e.g. known inducibility in embryos of various other P450s by "MC-type" inducers [15], our less than expected conceptal induction of EROD by MC [8–10]—based on a large number of past investigations of MC induction in numerous other extrahepatic tissues [16], and low rates of MC-induced conceptal EROD activities when compared with a profound MC-elicited induction in the same conceptal tissues as assessed with benzo[*a*]pyrene or AAF as substrates [8–10]. The potential effect of maternal influences on the inducibility and expression of rat conceptal P450 isoform(s) also posed questions of potential importance.

Thus, the present study addresses primarily two issues: (1) the expression and inducibility of P4501A1 and/or closely related isoform(s) in the whole embryo culture system, a system free of the maternal influences extant in previous investigations in which *in utero* induction was effected [8–10], and (2) a more rigorous identification (via cDNA sequencing of the entire coding region) of an MC-inducible rat conceptal isoform detected by us in this and earlier investigations. In addition, we investigated the capacity of other "MC-type" inducing agents, BNF and BA, to induce the conceptal P450 isoform *in vitro* in the whole embryo culture system, and studied, preliminarily, the capacity of a glucocorticoid, DEX, to influence P4501A1 expression and inducibility in conceptal tissues of the same system.

MATERIALS AND METHODS

Chemicals. MC, BA, BNF, NADPH, glucose 6-phosphate and DEX were purchased from the Sigma Chemical Co. (St. Louis, MO). Resorufin and ANF were purchased from the Aldrich Chemical Co. (Milwaukee, WI). Methoxy-, ethoxy-, pentoxy- and benzyloxyresorufin ethers (also referred to as phenoxazone ethers) were synthesized in our laboratories according to methods described by Mayer *et al.* [17]. Resorufin and its ethers were purified (> 99.5%) by the methods described by Klotz *et al.* [18]. Spectrophotochemical grade DMSO was purchased from the J.T. Baker Chemical Co. (Phillipsburg, NJ). All other chemicals utilized were reagent grade or better and were obtained from commercial sources.

Embryo culture. Time-mated pregnant Sprague–Dawley rats were obtained from Tyler Laboratories (Redmond, WA). The pregnant animals were delivered to and housed in the University of Washington vivarium 3–4 days prior to explantation of embryos and were given free access to food and water. Embryos were explanted on day 9.5 of gestation; the morning after copulation was defined as day 0, i.e. the beginning of gestation. Explantation procedures and embryo culture conditions were as described in detail previously [19]. Briefly, after explantation on day 9.5, embryos were cultured for 18 hr, after which the embryos were staged as described earlier [20]. Embryos not meeting the staging criteria [8–10 somites, partially elevated (~ 45°) neural folds and full dorsoflexion of the caudal region] were discarded. The incubation medium was changed, and the remaining embryos were then placed in culture bottles in accordance with their respective treatment schedules and cultured with fresh medium for an additional 30 hr.

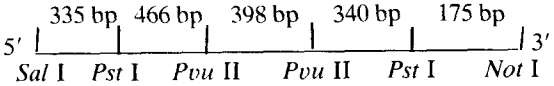
Inducing agents (MC, BNF or BA), alone or in combination with DEX, were dissolved in a maximum of 20 μL DMSO and were added to 14 mL of culture medium immediately after conceptal explantation (at the beginning of the culture period). The same chemicals, at the same concentrations, were added again when the culture medium was changed, after the initial 18 hr culture period. After treatment for 48 hr, embryos were removed from the culture medium and, in certain experiments, carefully evaluated for growth and development according to procedures previously described [21] and afterwards processed for measurements of enzymic activity as described below. In other experiments, the cultured embryos were rapidly evaluated microscopically for suboptimal growth and development and then homogenized immediately in guanidinium thiocyanate buffer to prevent RNA breakdown. RNA was isolated subsequently by phenol-chloroform extraction and further processed and analyzed as described below.

Enzyme assays. EROD, MROD, PROD and BZROD activities were assayed fluorimetrically as described previously [8–10] with a minor modification to include a 0.1 M Tris base buffer at pH 7.8 [22]. For these experiments, at least 10 cultured rat conceptuses (including the embryo proper, visceral yolk sac and amniotic membrane—ectoplacental cones were removed) were homogenized in 0.1 M Tris buffer (10–15 conceptuses per mL buffer), and the homogenate was centrifuged at 600 *g* for 5 min to remove cellular debris. The supernatant fractions were utilized as enzyme source, and generation of resorufin was assayed continuously as a function of time at fluorescence excitation and emission wavelengths of 530 and 585 nm, respectively. Enzymic activities were expressed as picomoles per milligram protein per minute with protein concentrations determined by the method of Lowry *et al.* [23].

RNA preparation, first strand cDNA synthesis, PCR amplification of cDNA and Southern blotting. For each experiment, tissues from a minimum of 30 cultured conceptuses were combined and processed as described previously [10]. Total RNA was extracted from conceptal homogenates as described by Chomczynski and Sacchi [24], and mRNA was isolated with a Poly(A+) Quick mRNA purification kit (Stratagene, La Jolla, CA). Reverse transcription of rat conceptal mRNA was performed with oligo

dT_{15} (Promega, Madison, WI) and AMV reverse transcriptase (Life Sciences, Inc., St. Petersburg, FL) as described previously [10]. The PCR sense primer utilized was 5′-GGAGTCGAC(*Sal* I)-GGGGGTAGTCCTTGCAGCTT-3′ and was designed to flank the 6th base of the 1st exon of the P4501A1 gene (*CYP*1A1). The antisense primer was 5′-TAGGCGGCCGC(*Not* I)-TCTGGTGAGCATCCAGGACA-3′ and was designed to flank the 3′-noncoding region of the 7th exon immediately downstream from the coding region. RT-PCR reactions were performed as detailed earlier [10] with a DNA thermal cycler (Perkin-Elmer Cetus, Norwalk, CT). The PCR-amplified cDNA was electrophoresed on 1.5% agarose gels and stained with ethidium bromide for visualization of an anticipated 1714 bp product that would include the entire coding region of *CYP*1A1 or a closely related isoform(s). DNA standards of known base length were co-electrophoresed with each sample. Electrophoresed cDNA was denatured as described by Sambrook *et al.* [25], transferred to nitrocellulose membranes for Southern blots as described by Schatz [26] and hybridized with 5′-AGCCTGGAGATGCTGAGGAC-3′ as an internal 20-mer oligonucleotide probe [27]. The nitrocellulose membranes were then washed and exposed to XAR Kodak X-ray film with intensifying screens (Fisher Scientific, Seattle, WA) for varying times as previously described [10].

Cloning, subcloning and sequencing of RT-PCR-amplified cDNA. The RT-PCR-amplified cDNA was cleaved with *Sal* I and *Not* I restriction enzymes, and the resultant cDNA was ligated into *Sal* I/*Not* I-cleaved Bluescript vector (Stratagene Cloning Systems, La Jolla, CA) in accordance with methods described in the Stratagene manual. The plasmids containing the putative 1714 bp insert were then cleaved as follows:



The cleaved cDNA fragments were isolated by separation on low melting point agarose gels, and each fragment was then ligated separately into Bluescript vector with the corresponding restriction sites. cDNA from both strands of each fragment was sequenced utilizing the Sequenase 2 System (U.S. Biochemical Life Science Co., Cleveland, OH) and a Taq Dyedeoxy Terminator Cycle Sequencing Kit (Applied Biosystems, Foster City, CA).

Statistics. The statistical significance of differences in treatment effects was assessed with ANOVA followed by Dunnett's multiple range test. The level of significance chosen was $P < 0.05$. All statistical procedures are described by Zar [28].

RESULTS

Inducibility in vitro *by MC.* In range-finding experiments, we assessed the effects of additions of MC to the culture medium at initial concentrations of 0.025, 0.25, 2.5 and 25 μM. Conceptal EROD activities were monitored for indications of P450 induction. Concentrations of 0.025 and 0.25 μM MC produced no indications of significant induction, and solubility limits for MC in the culture medium were slightly exceeded at 25 μM as judged from observance of traces of undissolved MC crystals. At the 2.5 μM concentration, a 2- to 3-fold increase in EROD activity was observed. Thus, in subsequent experiments with MC as inducer, concentrations of 2.0, 5.0 and 20 μM MC were utilized, and the effects of these concentrations on EROD activities of cultured conceptuses are presented in Table 1. The greatest increases in EROD activities were observed after additions of 20 μM MC, and these increases were approximately 6- to 7-fold in the first set of experiments. Comparable induction (3- to 8-fold) was observed following treatment of conceptuses *in utero* [8, 9] and also when embryos were exposed for only the first 18 hr period immediately after embryo culture was initiated, i.e. when MC was omitted from the second culture medium change (results not shown). At 1.0 and 10 μM concentrations, ANF inhibited induced EROD activities by 80 and 87%, respectively, but 10 μM ANF produced no significant effect on basal EROD activities (Table 1). MROD activities were below the level of detectability (< 0.1 pmol/mg protein/min) in reaction vessels containing enzyme sources from both control and MC-induced conceptuses. PROD and BZROD activities were detectable in reaction vessels containing 600 *g* × 5 min supernatant fractions from both control and MC-treated conceptuses, but MC treatment produced only minor and statistically insignificant increases (< 2-fold) in specific activities (results not shown). MC did not produce detectable dysmorphogenic or other embryotoxic effects (e.g. decreased embryo size, decreased embryonic protein content, and increased embryonic mortality) at any of the concentrations tested.

Inducibility in vitro *by BNF and BA.* In separate experiments, additions of 5, 20 and 100 μM BNF or BA to the culture medium were directly compared

Table 1. Induction by 3-methylcholanthrene (MC) and inhibition by 7,8-benzoflavone (ANF) of 7-ethoxyresorufin *O*-deethylase (EROD) in cultured rat conceptuses

MC* (μM)	ANF† (μM)	EROD‡ (pmol/mg/min)
0 (control)	0	0.18 ± 0.02
2	0	0.47 ± 0.12§
5	0	0.54 ± 0.07§
20	0	1.22 ± 0.36§
20	1	0.24 ± 0.04
20	10	0.16 ± 0.03
0	10	0.22 ± 0.05

* Final concentrations of MC in the culture medium. Controls received equal volumes of DMSO vehicle (see Materials and Methods).

† Final concentrations of ANF in assays of EROD activities.

‡ Values are means ± SD of 3–4 assays.

§ Differences from the control value were statistically significant (ANOVA) at $P < 0.05$.

Table 2. Effects of P4501A1 inducers on 7-ethoxyresorufin *O*-deethylase (EROD) activities in cultured rat conceptuses

Inducer	Concentration* (μM)	EROD† (pmol/mg/min)
Control		0.12 ± 0.06
3-Methylcholanthrene	20	1.53 ± 0.14‡
5,6-Benzoflavone	5	0.64 ± 0.21‡
5,6-Benzoflavone	20	1.32 ± 0.17‡
5,6-Benzoflavone	100	2.50 ± 0.46‡
Benz[*a*]anthracene	5	0.51 ± 0.09‡
Benz[*a*]anthracene	20	1.29 ± 0.23‡
Benz[*a*]anthracene	100	0.15 ± 0.06

* Final concentrations of inducers in the culture medium. Controls received equal volumes of DMSO vehicle (see Materials and Methods).

† Values are means ± SD of 3–4 assays.

‡ Differences from the control value were statistically significant (ANOVA) at $P < 0.05$.

Table 3. Combined effects of dexamethasone (DEX) and 3-methylcholanthrene (MC) on ethoxyresorufin *O*-deethylase (EROD) activities in cultured rat conceptuses*

MC (μM)	DEX (μM)	EROD† (pmol/mg/min)
0	0	0.12 (0.11–0.13)
0	2.5	0.15 (0.13–0.17)
0	10.0	0.13 (0.09–0.17)
20	0	0.73 (0.69–0.77)
20	2.5	0.79 (0.72–0.86)
20	10.0	0.82 (0.73–0.91)

* MC and DEX were placed in the culture medium at the onset of the culture period and after 18 hr at the concentrations indicated, as described in Materials and Methods. Controls received equivalent volumes of DMSO vehicle.

† Specific activities are the means of replicate values. Ranges are in parentheses.

with additions of 20 μM MC for effects on inducibility, as assessed by measurements of increases in EROD activities; the results are presented in Table 2. Increases in EROD activities produced by 20 μM MC were somewhat greater (approximately 13-fold) in this set of experiments than in the previous (Table 1) experiments, due in part to lower observed basal levels of activity. Additions of 20 μM BNF or BA each elicited approximately 11-fold increases in EROD activity. These increases were comparable to the approximately 13-fold increase produced by MC at the same concentration. BNF was roughly twice as effective at the 100 μM concentration as at the lower (20 μM) concentration of the same chemical for enzyme induction, but no induction by BA was evident at the highest (100 μM) concentration, possibly due to embryotoxicity (see Discussion). The effects of BA or BNF at concentrations higher than 100 μM were not evaluated because the solubility limits of these chemicals in the incubation medium were exceeded at higher concentrations. No indications of dysmorphogenesis (anatomical defects) were detected at any of the concentrations of BNF, BA or MC under investigation although inclusion of 100 μM BA or BNF in the culture medium did result in underdeveloped, growth-retarded embryos as assessed from measurements of maximal embryonic length and protein. The results obtained with BNF and BA were confirmed in repeated experiments.

Effects of DEX on EROD activity and inducibility. The effects on conceptal EROD activities of additions to the culture medium of various concentrations of DEX (alone and in combination with 20 μM MC) are presented in Table 3. Although the capacity of MC to produce marked increases in conceptal EROD activity was again clearly evident in these experiments, concomitant additions of 2.5 or 10 μM DEX to the culture medium produced no observable effect on the magnitude of MC induction, nor did the same concentrations of DEX produce any detectable effect on basal levels of conceptal EROD activity when added alone. Combinations of MC (20 μM) and DEX at concentrations higher than 10 μM resulted in a high incidence of markedly underdeveloped and grossly malformed embryos. At the concentrations of DEX (2.5 and 10 μM) in combination with MC (20 μM) used in the experiments reported in Table 3, detectable dysmorphogenic or embryotoxic effects did not differ statistically from the normal controls.

Detection of induced transcripts and sequencing. In experiments with northern blot analyses, P4501A1 mRNA signals could not be detected in cultured conceptuses treated with 20 μM MC. These results were consistent with previously reported data after treatment with MC *in utero* in which increases in levels of P4501A1 mRNA likewise could not be detected with northern blot assays. (The same 20-mer, P4501A1-specific oligonucleotide previously described was used as a probe.) However, RT-PCR with cytochrome P4501A1 cDNA (*CYP*1A1)-specific primers [10] produced sufficient material to obtain an ethidium bromide-stained cDNA band of expected size (1.7 kb) on agarose gels (Fig. 1A) for cultured conceptuses in which induction had occurred as judged from increased EROD activities. No corresponding ethidium bromide band was detected in conceptuses that were noninduced as judged by the lack of increased EROD activities. Also, no signals were obtained in induced conceptuses when reverse transcriptase was omitted from reverse transcription reaction mixtures. Experiments with Southern blotting with a 20-mer, ^{32}P-labeled internal hybridization probe [10] selective for *CYP*1A1 (Fig. 1B) also yielded results consistent with the concept that the amplified cDNA detected on the ethidium bromide stained agarose gels after RT-PCR was complementary to adult rat P4501A1 mRNA.

In addition to the above investigations, we wished to determine whether the complete cDNA coding sequence for the conceptal cytochrome was, in fact, identical to that reported for the hepatic P4501A1 isoform from induced adult rats. Results of the cloning and sequencing experiments (described in Materials and Methods) indicated an exact identity with the sequence reported by Hines *et al.* [29]. The

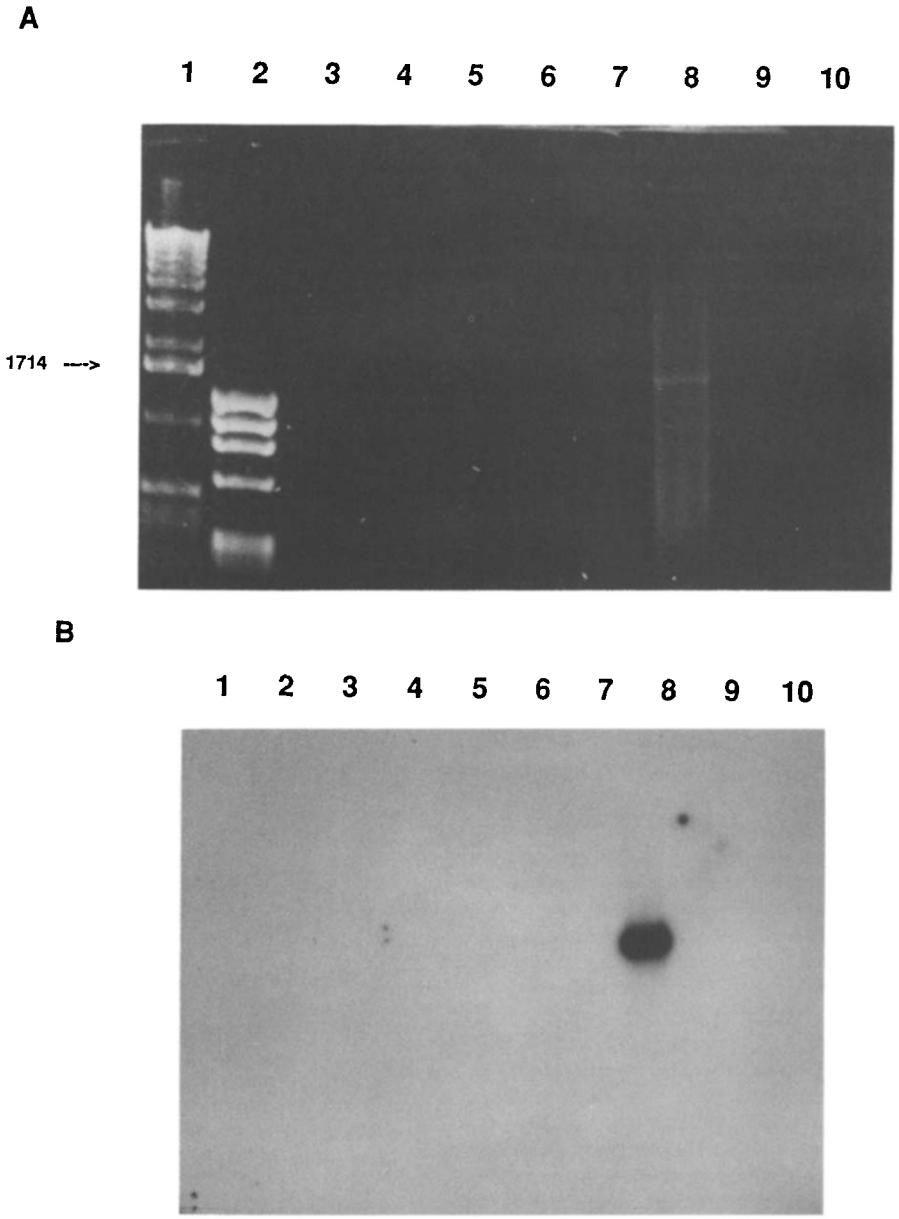


Fig. 1. RT-PCR products generated from mRNA obtained from cultured rat conceptuses exposed to 20 μM MC or to vehicle (DMSO). Primers were selective for P4501A1 (see Materials and Methods). (A) Ethidium bromide stained agarose gel shows RT-PCR products obtained by amplification of cDNA synthesized from oligo-dT selected mRNA of conceptal tissues. PCR reaction mixture (5 μL) was loaded onto the gel. Lanes 1 and 2, DNA size markers; lane 3, blank; lane 4, mRNA from MC-treated embryos but without reverse transcriptase in the reverse transcription reaction mixture; lane 5, blank; lane 6, mRNA from vehicle-treated embryos; lane 7, blank; lane 8, mRNA from MC-treated embryos; lane 9, blank; lane 10, 1st strand cDNA was omitted from the PCR reaction mixture. The arrow indicates the migration position of the RT-PCR product at a 1714-bp size as predicted from usage of the described primer pair. (B) Southern blotting of the same gel. Hybridization was performed with a ^{32}P-labeled internal probe (see Materials and Methods).

sequencing process was performed on both sense and antisense strands for purposes of confirmation, and a representative sequence is depicted in Fig. 2.

DISCUSSION

The results of the current investigations indicate that marked induction of conceptal P4501A1 can be achieved *ex utero* in the whole embryo culture system. This culture system is now employed extensively in studies of the dysmorphogenic and other embryotoxic effects of chemicals; thus, the induction of P4501A1 demonstrated here may be particularly relevant to investigations in which

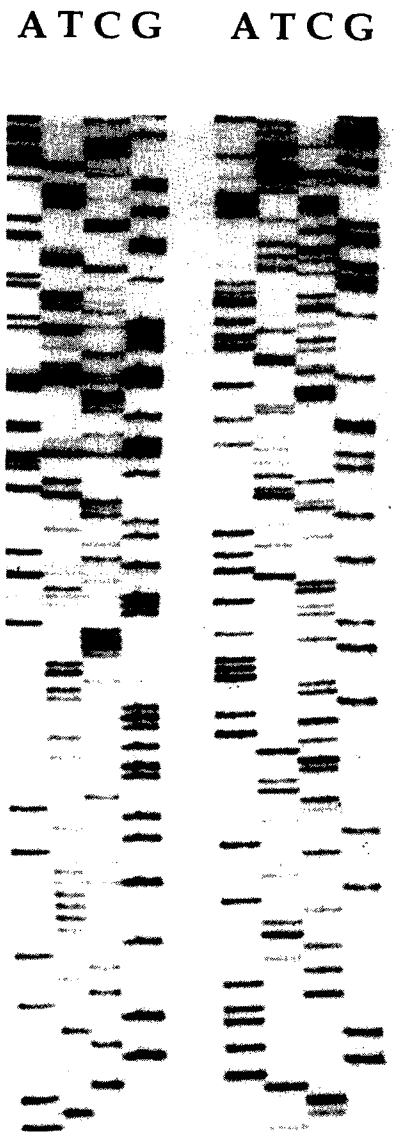


Fig. 2. Representative sequence of cDNA generated in the RT-PCR reactions (see Materials and Methods). The sequence illustrated was from the subcloned *Sal* I/*Pst* I fragment.

chemicals under investigation are substrates for, or modulators, of P4501A1.

Of interest is the observation that the magnitude of induction achieved *ex utero* in these studies was comparable to the magnitude of induction reported following exposures *in utero* [8–10]. In neither case can it be assumed that maximal induction was achieved because of the impracticality of assessing all possible variables of timing and dosage. The fact that EROD activities were highest at the highest (solubility-limited) concentrations tested *ex utero* for MC and BNF suggests, in fact, that maximal induction was not achieved. A decrease observed in EROD activities at the highest tested levels of BA (100 μM) remains unexplained, but a similar effect has been observed in cultured mouse embryo cells [30], and cytotoxic effects remain a possible cause. Nevertheless, the results suggest quite strongly that the apparently low level of P4501A1 inducibility observed in conceptal tissues *in utero* (in comparison with the very high "MC-type" inducibility that has been observed frequently in other extrahepatic tissues by several investigators in numerous previous investigations [16]) was not due to limitations by maternal factors or to inaccessibility of the inducing agent to conceptal tissues/cells. Rather, the relatively low inducibility of P4501A1 in conceptal tissues *in utero* would appear to be a function of regulatory factors present within the conceptal cells *per se*. The identity and mode(s) of action of such regulatory factors operating within conceptal tissues remain to be elucidated, but could include a deficiency in expression of one or more components of the system mediating *CYP*1A1 induction, i.e. cytosolic Ah receptors, hsp 90 proteins, Arnt proteins, or various other factors [31]. Such factors may be intimately associated with cellular differentiation and proliferation. The current investigations provide evidence that all of the elements involved in P4501A1 induction are both present and functional in conceptal tissues during the stage of organogenesis.

The present results demonstrate that in addition to MC, BNF and BA were also effective inducers of conceptal P4501A1 during organogenesis. This suggests the likelihood that all "MC-type" inducing agents (Ah receptor agonists) would be capable of the same inducing activity during organogenesis. MC and BA are prototypic of the polynuclear aromatic hydrocarbons, a well-known class of "MC-type" inducing agents, and BNF is a prototype for the flavones, also effective P4501A1/2 inducing agents. Other classes of agents known to behave as "MC-type" inducers include the dioxins and structurally related chemicals such as the dibenzofurans, azo- and azoxybenzenes, etc. (TCDD is a prototype), planar polyhalogenated biphenyls (3,4,5,3′,4′,5′-hexachlorobiphenyl is a prototype), methylated xanthines (caffeine is a prototype), certain indoles (indolo[3,2-*b*]carbazol is a prototype) and phenothiazines. These chemicals also may be expected to induce P4501A1 in rat conceptal tissues during organogenesis although the effectiveness and magnitude of induction will depend upon a large variety of factors including dosage, potency and accessibility *in utero*. The effects of the recently described P4501A1 inducers omeprazole and mevinolin on conceptal P4501A1 levels may be of interest since, unlike the inducers discussed above, the mechanisms of induction for mevinolin and omeprazole were suggested to be independent of binding to the Ah receptor [32, 33]. Cycloheximide regulation of P4501A1 induction in cultured embryos may also be of interest since cycloheximide regulation of induction may be limited to culture-adapted cells [34].

Ample precedent has been set for the conceptal-specific expression of genes coding for various hemoproteins. The γ-chain of fetal hemoglobin represents a classical example, and conceptal-specific P450 expression is also well known. In humans, the gene coding for P4503A7 (*CYP*3A7) is expressed in

hepatic tissues prenatally but not postnatally, whereas a closely related gene, *CYP*3A4, is expressed postnatally but not prenatally [35]. The physiologic and toxicologic implications of the specific prenatal expression of genes coding for certain P450 hemoproteins are not yet fully understood. The investigations reported here provide evidence that a similar situation does not exist with respect to *CYP*1A expression in the rat. Sequence data indicate that the induced isoform expressed in rat conceptal tissues during organogenesis is identical to an inducible P4501A1 isoform expressed in adult rat hepatic tissues [29]. Although we did not specifically search for a P450 species similar to the TCDD-inducible murine isoform discovered recently [11–13], expression of such an isoform(s) in rat conceptal tissues during organogenesis now seems somewhat unlikely. However, we cannot conclusively exclude the possibility at this time. Likewise, expression of functional P4501A2 in rat tissues during organogenesis seems quite unlikely at present. The selective capacity of rat P4501A2 to catalyze the O-demethylation of methoxyresorufin [14, 36] coupled with the lack of detectability of MROD activity after MC induction of EROD in these and previous experiments [8–10] argue that functional P4501A2 is not expressed in conceptal rat tissues during organogenesis, even after exposure of conceptuses to levels of "MC-type" inducing agents that produce marked increases in conceptal P4501A1. Lack of sequence information for the P450MCX isoform recently reported by Weaver *et al.* [14] precludes speculation pertaining to sequence homology to the P4501A1 isoform, and we do not know if our procedure would have detected this isoform in the rat conceptus.

Several investigations have demonstrated the capacity of glucocorticoids to enhance P4501A1 induction by "MC-type" inducers in various systems *in vitro* as well as *in vivo* [e.g. 37, 38], although a recent study in rainbow trout has indicated a profound negative regulatory effect of glucocorticoids on *CYP*1A1 expression [39]. A putative glucocorticoid responsive element located in the 1st intron of *CYP*1A1 [37] or glucocorticoid-enhanced production of the Ah receptor [40] may contribute to such effects. In the investigations reported here, no significant effect of DEX on expression of conceptal *CYP*1A1 or its induction by MC was observed. Investigations at higher glucocorticoid concentrations were not feasible due to the pronounced embryotoxicity observed with combinations of MC and DEX at the higher DEX concentrations, and therefore glucocorticoid effects on regulation of conceptal *CYP*1A1 expression and induction cannot be ruled out entirely.

In summary, the results presented demonstrate that cytochrome P4501A1 can be induced both by polycyclic aromatic hydrocarbons and by benzoflavones in cultured whole embryonic rat tissues during organogenesis, a period in prenatal development that is highly susceptible to the dysmorphogenic and other embryotoxic effects of chemical agents. These results strongly suggest that other Ah receptor agonists would also effect induction in the same system. Maternal factors appeared to play only a minor role as determinants of the magnitude of induction elicited. We were unable to demonstrate an effect of the glucocorticoid DEX on the magnitude of induction by MC or on expression of *CYP*1A1 in the absence of an inducing stimulus. Sequence analysis of the cDNA coding region of the induced isoform indicated that the induced conceptal P450 was identical to P4501A1 induced by Ah receptor agonists in adult rat hepatic tissues.

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