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Pulmonary CYP1A1 and CYP1A2 levels and activities in adult male and female offspring of rats exposed during gestation and lactation to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin

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Abstract

The levels and activities of pulmonary microsomal CYP1A1 and CYP1A2 in 40-day-old male and female, and 120-day-old male offspring of pregnant rats treated with five weekly 0.1 μg/kg doses of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) during gestation and lactation were compared with those in age-matched offspring of untreated dams. The CYP1A1-preferential activity, ethoxyresorufin *O*-deethylase (EROD), was comparably induced 5.3- and 6.4-fold in 40-day-old male and female offspring, respectively, but was not induced in 120-day-old male offspring, of TCDD-treated dams. Similarly, CYP1A1 protein was induced in 40-day-old female or male offspring of untreated dams but was undetectable in 120-day-old offspring of untreated or treated dams. CYP1A2 activity, as measured by the bioactivation of 2-amino-3,4-dimethylimidazo[4,5-*f*]quinoline (MeIQ) to mutagens in the Ames assay, was elevated 11.1- and 5.5-fold in 40-day-old female and male offspring, respectively, of TCDD-treated dams, but was unaffected by TCDD exposure in 120-day-old offspring. CYP1A2 protein was undetectable in 40-day-old male or female offspring of untreated dams or in 120-day-old male offspring of treated or untreated dams; it was detected in 40-day-old offspring of treated dams, at a level that was higher in females than in males. The results show that gestational and lactational exposure to TCDD causes long-lasting and gender-preferential induction of CYP1A1 as well as CYP1A2 in the lungs of rat offspring.

Keywords: Lung microsomes; CYP1A1; 2 induction; EROD activity; MeIQ bioactivation; Gestational TCDD exposure

1. Introduction

CYP1A1 induction is among the most prominent and best characterized effects of TCDD [1,2]. Enhanced catalytic activities resulting from the induction of CYP1A1 have been implicated in several of the adverse health effects of this polyhalogenated hydrocarbon [3,4], which include teratogenicity and other developmental defects [5–7]. The enzyme is of carcinogenic importance because it catalyzes the bioactivation of many procarcinogenic polyaromatic hydrocarbons (such as B[a]P [8–10]) and certain heterocyclic aryl amines (such as PhIP) [11] to their ultimate reactive intermediates. These activities of the enzyme have also been implicated in other disorders, such as cardiovascular diseases [12,13].

CYP1A1 induction by TCDD is mediated by the binding of TCDD to the AhR. Following TCDD binding, the resulting ligand–receptor complex translocates to the nucleus and binds to a nuclear transcription factor, ARNT [1–3,14]. The AhR–ARNT complex then binds to consensus sequences in the regulatory domains of the *CYP1A1* gene [1–3,14]. Other target genes of the AhR–ARNT complex include those encoding other enzymes of endogenous and foreign compound metabolism, including CYP1A2 [1–3,14]. Thus, expression of CYP1A1 and CYP1A2 is commonly coordinately regulated by many chemicals. However, CYP1A2 expression may be regulated independently of CYP1A1 expression [15–18]. Both enzymes differ also in their tissue distribution, with CYP1A1 expression reported to be preferentially extrahepatic, and CYP1A2 expression preferen-

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Abbreviations: AhR, aryl hydrocarbon receptor; ARNT, AhR nuclear translocator; AUC, area under the curve; B[a]P, benzo[*a*]pyrene; CYP1A, used to include CYP1A1 and CYP1A2; EROD, ethoxyresorufin *O*-deethylase; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; MeIQ, 2-amino-3,4-dimethylimidazo[4,5-*f*]quinoline; PCR, polymerase chain reaction; PhIP, 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine; and TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin.

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tially hepatic [19,20]. However, a few studies have reported the inducibility of CYP1A2 in extrahepatic tissues, including the lungs of experimental animals [16,21–24]. CYP1A2 is of toxicological interest as well because it catalyzes the bioactivation of many promutagens and procarcinogens including aryl amines, food-derived heterocyclic aryl amines, and tobacco-specific nitrosamines [11,25,26].

Several studies have examined the effect of gestational and lactational TCDD exposure on the levels and activities of CYP1A in the liver. We reported previously that hepatic CYP1A1 but not CYP1A2 is induced in 25-day-old prepubertal but not 120-day-old adult offspring of rats treated subcutaneously during gestation and lactation with a cumulative 0.5 μg/kg dose of TCDD [27]. Another study [28] reported that subcutaneous administration of a single 2.5 μg/kg dose of the compound to pregnant rats causes a 100-fold induction of hepatic AHH activity, as well as extensive damage to the liver in the fetus. Other investigators [29] showed that oral administration of a single 3 μg/kg dose of the compound to pregnant rats causes a 20-fold induction of hepatic AHH activity in the fetus. In the latter study [29], the activity remained elevated through postnatal day 21, at which time the induction was 3- to 5-fold the activity in controls.

The effect of gestational and lactational TCDD exposure on the levels and activities of CYP1A1 in extrahepatic tissues has not been investigated extensively. Such studies are of toxicological importance because target tissue-mediated metabolic bioactivation may play a dominant role in the toxic effects of a chemical in an extrahepatic tissue. The objective of the current study was to determine the level and activity of pulmonary CYP1A1 in adult offspring of rats exposed during gestation and lactation to low doses of TCDD. The results demonstrated that the exposure causes induction of CYP1A1 in the lungs, which may be more pronounced and longer-lasting than previously observed in the liver [27]. In addition, we report the novel and incidental observation that exposure to TCDD induces CYP1A2 in the lungs, an effect that was not observed previously in the liver [27].

2. Materials and methods

2.1. Materials

The following were obtained from the commercial sources indicated in parentheses: ethoxyresorufin (Pierce Chemical Co.); sodium resorufin (Molecular Probes); MeIQ (Midwest Research Institute); ECL western blotting analysis system (Amersham Life Sciences); Zeta-Probe nylon membrane (Bio-Rad Laboratories); D-biotin and L-histidine (Curtis Mathesson Scientific, Inc.); glucose-6-phosphate dehydrogenase (Boehringer-Mannheim); Agar Noble (Difco Laboratories); nitrocellulose membranes (Schleicher & Schuell); [α-^{32}P]ATP (Dupont-NEN); Kodak Biomax MR and X-Omat AR imaging films (Fisher Scientific Co.); plasmid pmP3450–3′ (American Type Culture Collection); RETROscript PCR Kit (Ambion); AmpliTaq Gold (Perkin Elmer Biosystems); GAPDH primers (synthesized and purified by Sigma-Genosys); rat CYP1A1 and CYP1A2 primers (synthesized and purified by The Robert Wood Johnson Medical School DNA Laboratory); and 100 bp DNA ladder (New England Biolabs). TCDD was provided by Dr. Michael Gallo, University of Medicine and Dentistry of New Jersey, Robert Wood Johnson Medical School; plasmid p210 was provided by Dr. John Fagan, Maharishi International University; CD5 and human GAPDH cDNA were provided by Drs. Paul Thomas and Suzie Chen, respectively, Rutgers University; and *Salmonella typhimurium* TA98 was provided by Dr. Bruce Ames, University of California. All other reagents and supplies used were of the highest grade of purity.

2.2. Animals and pretreatment

Timed-pregnant Sprague–Dawley rats (Taconic Farms), weighing 220–240 g at the initiation of the experiments, were housed individually in polypropylene shoebox cages on wood shavings for bedding and given free access to food and water. Colony room lights were set on a 12-hr light–dark cycle. Four dams were injected subcutaneously with 0.1 μg/kg of TCDD (dissolved in 100 μL of corn oil) once a week on days 7, 14, and 20 of gestation and on postpartum days 9 and 16, for a cumulative dose of 0.5 μg/kg. Two dams were treated with corn oil only, and three dams were untreated. The corn oil used was free of peroxides, as described previously [30]. Each liter was culled to a maximum of eight pups at birth. Body weight growth curves were recorded throughout the duration of the study, and liver-to-body weight ratios were determined at the termination of the study.

2.3. Preparation of total RNA and microsomes and analysis of the samples

On postnatal day 40 or 120, male or female offspring were euthanized, and their lungs were isolated asceptically and frozen immediately in liquid nitrogen prior to storage at −80°. Portions of each lung were excised for the isolation of total RNA by the method of Chomczynski and Sacchi [31]. Washed hemoglobin-minimized microsomes were prepared from each remaining lung by differential centrifugation as described previously [32] except that autoclaved (sterile) phosphate buffer (10 mM, pH 7.4), containing 1.15% KCl, was used for homogenization of the lung and washing of the microsomes.

Analyses of the samples were carried out in two sets of experiments. In the first set of experiments, pooled lung microsomes and pooled total RNA from individual rats were analyzed for CYP1A proteins and mRNAs, respectively, and lung microsomes from individual rats were used

for assays of EROD activity and MeIQ bioactivation. In the second set of experiments, lung microsomes and total RNA from individual animals were analyzed for CYP1A proteins and mRNAs, respectively.

2.4. EROD activity

Microsomal *O*-deethylation of ethoxyresorufin, a preferential activity of CYP1A1 [33,34], was assayed fluorometrically as described by Pohl and Fouts [35], at a substrate and protein concentration of 5 μM and 100 μg/mL, respectively.

2.5. Bioactivation of MeIQ to mutagens

Bioactivation of MeIQ to mutagenic derivatives, a CYP1A2-selective activity [11,25], was assessed by the Ames test, using *S. typhimurium* TA98 as the tester strain. We used a modification [36] of the preincubation method of Maron and Ames [37], in which microsomes were substituted for liver S9 as the source of activating enzyme. Briefly, a 0.1-mL aliquot of an overnight culture of *S. typhimurium* TA98 was incubated in the presence of NADPH (as an NADPH-generating system [36]), lung microsomes (0.25 mg protein), and MeIQ (0.75 μg in 5 μL DMSO), all in a final volume of 0.5 mL of phosphate buffer (0.1 M, pH 7.4). NADPH was omitted from control incubations. Following incubation with shaking at 37^o for 45 min, the mixture was combined with a 2-mL aliquot of molten top agar (containing 10% histidine and biotin), plated, and incubated at 37^o for 48 hr. Each determination was in triplicate. Quantification of histidine-positive revertant colonies and assessment of toxicity to the bacteria were as described previously [36].

2.6. Electrophoresis and Western blot analysis

Western blot analysis of microsomes was performed, following SDS–PAGE, as described previously [38], using a mouse monoclonal anti-rat CYP1A1 IgG, which also recognizes CYP1A2 [39]. Immunoreactive bands were detected using Enhanced Chemiluminescence according to the instructions of the manufacturer and were quantified by densitometry using a Bio Image IQ scanner. Each densitometric (AUC) value was linear with respect to the amount of protein analyzed.

2.7. Northern blot analysis

CYP1A1, CYP1A2, and GAPDH mRNAs were analyzed by northern blotting as described previously [38], using a rat CYP1A1-specific cDNA probe from plasmid p210 [15], a mouse CYP1A2 cDNA from plasmid p3450–3′, and a human GAPDH cDNA, respectively. Briefly, total lung RNA (20 μg) was fractionated on a denaturing formaldehyde/agarose (1%) gel and transferred to Zeta-Probe nylon membrane according to the recommendation of the manufacturer. ^{32}P-Labeling of the CYP1A1, CYP1A2, and GAPDH probes with [α-^{32}P]ATP by random priming, hybridization and washing of the filters, and autoradiography were as described previously [38]. Autoradiographic bands corresponding to CYP1A1 and GAPDH transcripts were quantified by densitometry, using a Bio Image IQ scanner. Densitometric values for CYP1A1 transcripts were normalized to those of corresponding GAPDH transcripts.

2.8. RT–PCR analysis

For the synthesis of the cDNA template, 1 μg of total RNA, 0.5 mM dNTPs, 5 μM oligo-dT primer, 10 U placental RNasin, and 100 U Maloney murine leukemia virus reverse transcriptase (all from Ambion) were added to a total volume of 20 μL in 1X RT buffer. The sample was incubated in a Perkin Elmer GeneAmp PCR System 2400 at 42^o for 1 hr, followed by denaturation at 92^o for 10 min.

For the amplification reaction, which was completed in the PCR system, the rat CYP1A1 and CYP1A2 primers described by Kim *et al.* [40], and the human GAPDH primers described by Rumsby *et al.* [41] were used. A 1-μL aliquot of the first-strand (cDNA template) reaction mixture was added to 100 ng each of CYP1A1, CYP1A2, and GAPDH primers, 0.125 mM dNTPs, 3.3 mM $MgCl_2$, and 0.5 U AmpliTaq Gold, in a total volume of 20 μL 1X PCR buffer. The sample was cycled for 1 min at 94^o for denaturation, 1 min at 50^o for annealing, and 3 min at 72^o for extension, for a total of 30 cycles. CYP1A1 and CYP1A2 transcripts were amplified in the same reaction mixture because in preliminary experiments to optimize the PCR conditions, we observed no differences in the level of the transcripts in reaction mixtures containing primers for either CYP1A1 or CYP1A2 only, or both. The PCR products were electrophoresed on a 1% agarose gel along with a 100 bp DNA ladder, and visualized by staining in ethidium bromide.

2.9. Other assays

Protein was determined by the method of Lowry *et al.* [42]. Differences between means in EROD activity were analyzed by one-way analysis of variance followed by Tukey's post hoc adjustment. Differences were considered significant at a P value of 0.05.

3. Results

Treatment with corn oil alone or TCDD did not affect body weights of the dams in comparison with untreated control dams, as reported previously [27]. Similarly, neither corn oil nor TCDD treatment affected the number of pups per litter, the ratio of male to female offspring, the birth weights or subsequent body weights of the pups, or the liver-to-body weight ratios in the offspring.

Table 1
Pulmonary microsomal EROD activity in offspring of untreated and TCDD-exposed dams

Maternal treatment	EROD activity (pmol resorufin formed/mg protein/min)		
	Postnatal age (days)		
	40		120
	Female	Male	Male
Control	19.2 + 3.1[a]	17.3 + 3.0[ab]	12.0 + 2.4[b]
TCDD	122 + 11.3[c]	91.0 + 12.4[d]	19.2 + 3.1[a]
	(6.4)	(5.3)	(1.6)

EROD activity was assayed fluorometrically as described in "Materials and methods". Each value is the mean (± SD) of determinations in microsomes from three rats. Values in parentheses are the magnitude of induction by TCDD treatment for each group.
[a,b,c,d] Values bearing different superscripts are significantly different from each other ($P < 0.05$).

3.1. Effect of TCDD exposure on pulmonary microsomal EROD activity

In preliminary experiments, EROD activity in 40-day-old or 120-day-old offspring of untreated rats was not significantly different from the activity in age-matched offspring of corn oil only-treated rats. Accordingly, offspring of untreated rats were used as control animals in subsequent experiments. In untreated 40-day-old rats, pulmonary microsomal EROD activity was comparable in males and females but was induced by TCDD treatment to a slightly greater extent in females (6.4-fold) than in males (5.3-fold) (Table 1). In 120-day-old male rats, the activity in controls was comparable to that in control 40-day-old males, but was induced slightly (1.6-fold) by TCDD exposure (Table 1).

3.2. Effect of TCDD exposure on pulmonary microsomal MeIQ bioactivation (CYP1A2 activity)

In untreated 40-day-old rats, MeIQ bioactivation was comparable in males and females (Table 2). TCDD exposure induced CYP1A2 activity extensively but to a greater extent in females (11.1-fold) than in males (5.5-fold) (Table 2). In 120-day-old male rats, bioactivation of the amine was slightly lower than that in untreated 40-day-old males but was induced 2.1-fold by TCDD exposure (Table 2).

3.3. Effect of TCDD exposure on CYP1A protein levels

In the first experiment in which pooled microsomes from the animals were analyzed, the level of CYP1A1 protein in 40-day-old female offspring of untreated rats (0.82 AUC Units) was 1.9-fold higher than the level in their male counterparts (0.43 AUC Units) but was induced by TCDD exposure to a greater extent in males (216-fold) than in females (92-fold) (Figs. 1A and 2A). CYP1A2 protein, in contrast, was undetected in untreated 40-day-old male or female animals but was present following TCDD exposure, at a level that was 3-fold higher in females than in males (Figs. 1A and 2A). In 120-day-old rats, neither CYP1A1 nor CYP1A2 protein was detectable in control or TCDD-ex-

Table 2
Bioactivation of MeIQ to mutagens in the Ames assay catalyzed by pulmonary microsomes from offspring of untreated and TCDD-treated rats

Maternal treatment	MeIQ bioactivation (revertant colonies/plate)		
	Postnatal age (days)		
	40		120
	Female	Male	Male
Control			
− NADPH	40 ± 10	36 ± 6	35 ± 6
+ NADPH	93 ± 5	83 ± 13	67 ± 6
	(53)[a]	(47)[a]	(32)[a]
TCDD			
− NADPH	35 ± 6	37 ± 7	38 ± 5
+ NADPH	624 ± 41	296 ± 64	99 ± 11
	(589)[b]	(259)[b]	(67)[b]

The Ames assay was performed as described in "Materials and methods" with an NADPH-generating system omitted (− NADPH) or included (+ NADPH) in the preincubation step. Each value represents the mean (± SD) of determinations in microsomes from three rats, each in triplicate. Each value in parentheses is the difference in the number of revertant colonies between incubations in the presence and absence of NADPH.
[a,b] The ratio of b to a (b:a) in each group is the fold increase in MeIQ bioactivation caused by TCDD treatment, and is 11.1, 5.5, and 2.1 in 40-day-old female, 40-day-old male, and 120-day-old male rats, respectively.

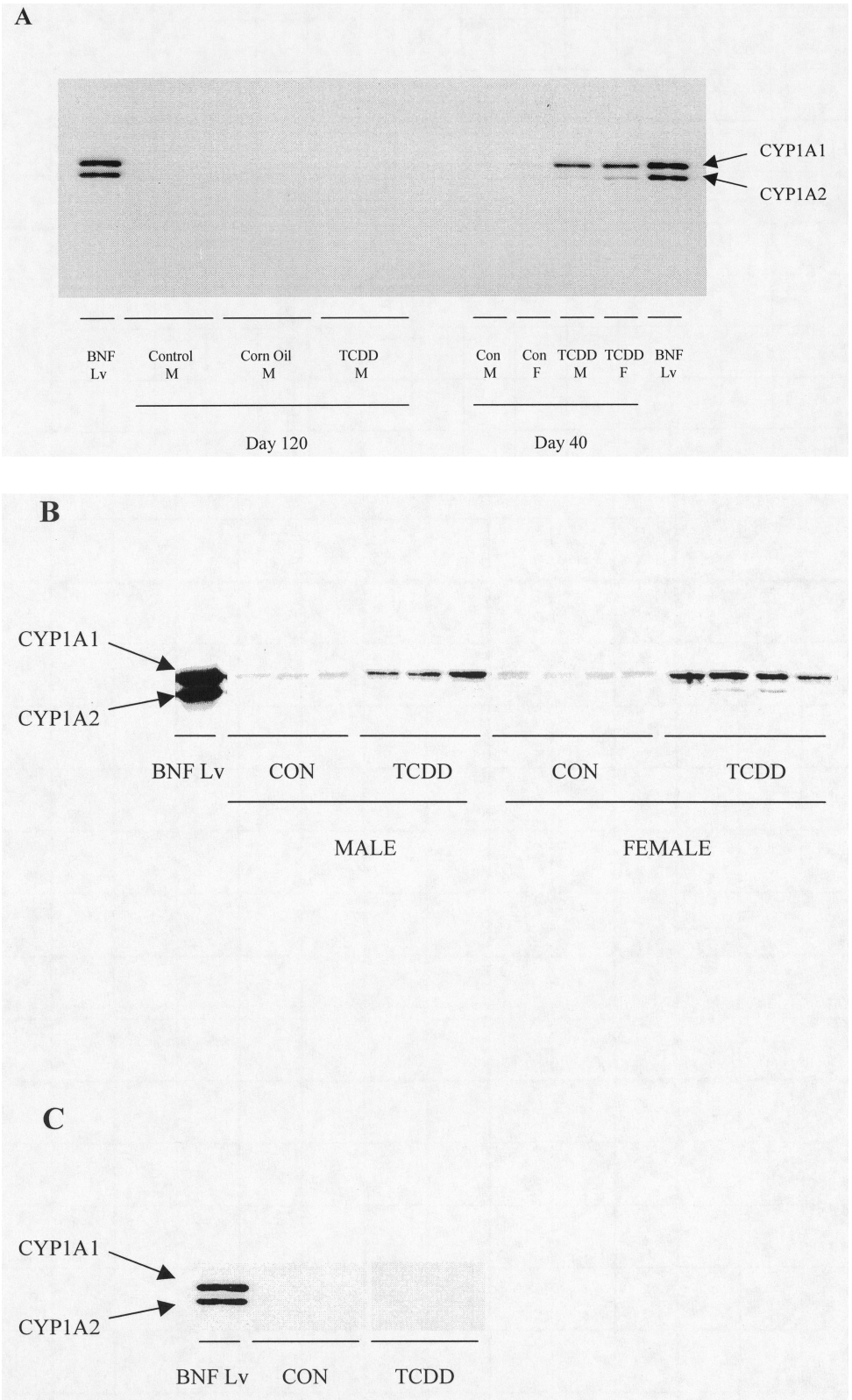

Fig. 1. Western blot analysis of pulmonary microsomal CYP1A from offspring of control and TCDD-treated dams. Lung microsomes (25 μg protein) were from 40-day-old (Day 40) or 120-day-old (Day 120) female (F) or male (M) offspring of untreated (Con), corn oil-treated (Corn oil), or TCDD-treated (TCDD) rats. (A) Each lane represents pooled microsomes from the lungs of three rats. (B, C) Each lane represents microsomes from individual 40-day-old rats (B) or 120-day-old rats (C), all different from those in panel A. Liver microsomes (0.25 μg protein) from β-naphthoflavone-treated rats (BNF Lv) were included as a positive control for CYP1A1/2. Electrophoresis, western blot analysis using CD5, a mouse anti-rat CYP1A1 monoclonal antibody that also recognizes CYP1A2, and detection by chemiluminescence were as referenced in "Materials and methods."

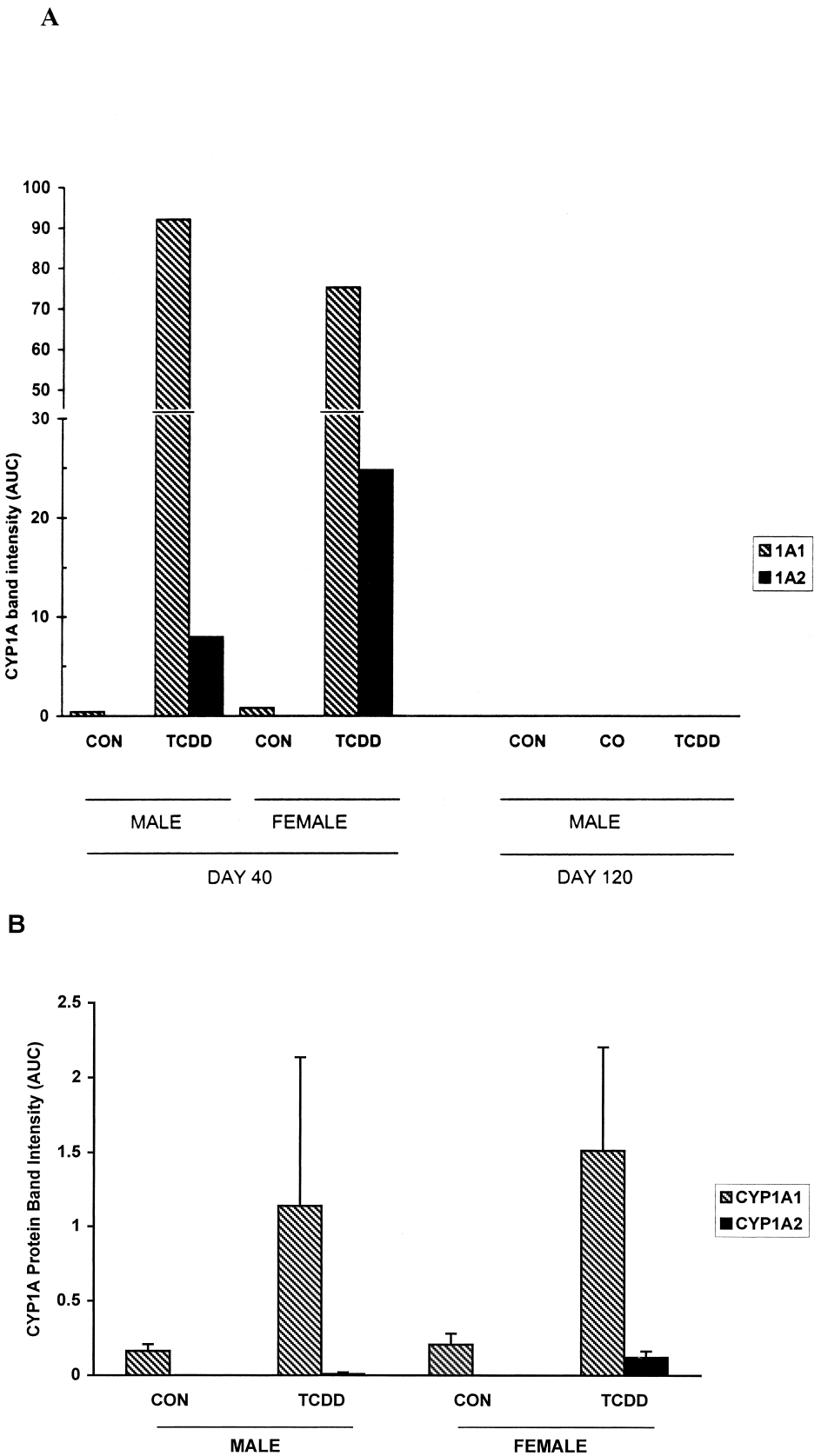

Fig. 2. Densitometric estimation of pulmonary microsomal CYP1A protein levels in offspring of control or TCDD-treated dams. Microsomes were from 40-day-old (DAY 40) or 120-day-old (DAY 120) offspring of control (CON), corn oil (CO), or TCDD-treated (TCDD) dams. (A) Values are from the western blots shown in Fig. 1A. Note the difference in the ordinate scale for CYP1A1 and CYP1A2. (B) Values are from the western blots in Fig. 1B. Each value is the mean (± SD) of 3 or 4 animals per group.

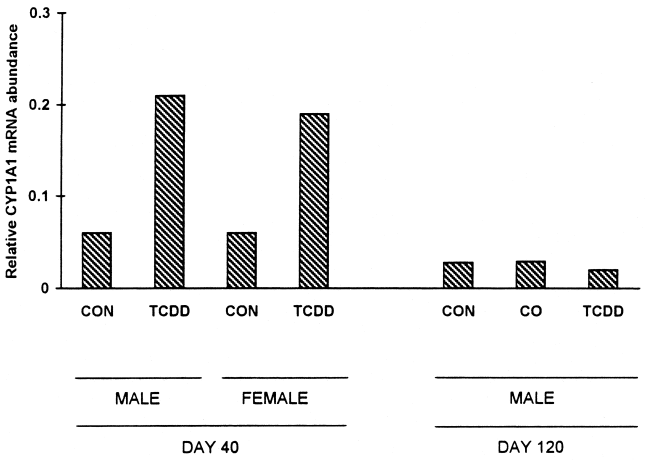

Fig. 3. Densitometric estimation of pulmonary CYP1A1 mRNA abundance in offspring of control, corn oil-treated, or TCDD-treated dams. Total lung RNA (20 μg) from 40-day-old offspring (DAY 40) or 120-day-old offspring (DAY 120) of untreated (CON), corn oil-treated (CO), or TCDD-treated (TCDD) dams were pooled from three rats and analyzed by northern blotting for CYP1A1 and GAPDH mRNA as described in "Materials and methods." Autoradiographic bands corresponding to CYP1A1 and GAPDH mRNA were quantified by densitometry. Relative CYP1A1 mRNA abundance is the amount of CYP1A1 mRNA relative to that of GAPDH mRNA.

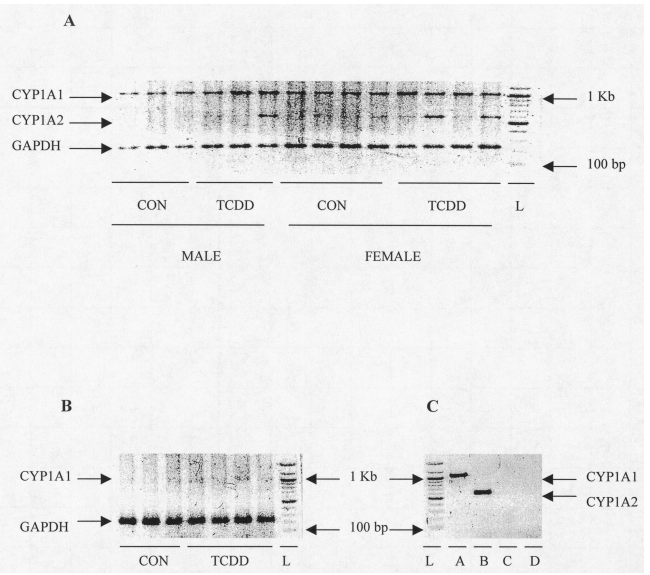

Fig. 4. RT–PCR analysis of CYP1A mRNA in 40-day-old (A) or 120-day-old (B) offspring of control (CON) or TCDD-treated (TCDD) dams. The data were obtained from the same animals used in Figs. 1B and 2B. PCR analysis was as described in "Materials and methods," using rat CYP1A1 and CYP1A2 primers described by Kim *et al.* [40] and GAPDH primers described by Rumsby *et al.* [41]. The amount of each amplification reaction mixture electrophoresed was 5 μL and 15 μL for the samples in panel A and panel B, respectively. The size of the CYP1A1, CYP1A2, and GAPDH PCR products was 1050, 600, and 240 bp, respectively. Lane L: 100 bp DNA ladder. Panel C: Each of the following was omitted from a complete reaction mixture containing total lung RNA from a 40-day-old TCDD-exposed female rat offspring: CYP1A2 primers (lane A), CYP1A1 primers (lane B), Taq polymerase (lane C), and cDNA template (lane D); 5-μL aliquots of the reaction mixture were electrophoresed.

posed animals at the level of microsomal protein analyzed (Figs. 1A and 2A).

The results of the second experiment, in which microsomes from individual animals were analyzed, show the inter-animal variability in the CYP1A1 and CYP1A2 induction responsiveness (Figs. 1B, 1C, and 2B). Overall, the results were comparable to those of the first set of experiments in which pooled microsomal samples were analyzed (Figs. 1A and 2A).

3.4. Effect of TCDD exposure on CYP1A mRNA levels

In the first set of experiments, in which pooled total RNA from the animals was analyzed by northern blotting, the level of CYP1A1 mRNA was comparable in 40-day-old offspring of untreated male and female rats, and was comparably elevated by TCDD exposure in males (3.5-fold) and females (3.1-fold) (Fig. 3). In 120-day-old male rats, the transcript was present at a comparable level in control and TCDD-exposed animals, but was only 30% of the level in 40-day-old control rats (Fig. 3). CYP1A2 mRNA was undetectable in any of the samples at the level of total RNA analyzed (data not shown).

In the second set of experiments, in which individual animals were examined, analysis of the transcripts by RT–PCR showed, in support of the northern blot data in Fig. 3, that male and female 40-day-old offspring of untreated dams had comparable levels of CYP1A1 mRNA as did male and female 40-day-old offspring of TCDD-exposed dams (Fig. 4A). The results also showed that the CYP1A1 transcript was present at very low levels in 120-day-old offspring of untreated or TCDD-treated dams (Fig. 4B). RT–PCR analysis of CYP1A2 mRNA revealed the presence of the transcript, in contrast with the findings with northern blotting. The CYP1A2 transcript was detectable, albeit at very low levels, in three of four 40-day-old female offspring of untreated dams but not in any of their three untreated male counterparts analyzed (Fig. 4A). The transcript was more abundant in 40-day-old female offspring of TCDD-exposed dams than in their untreated counterparts, and was detectable in only one of the three 40-day-old male offspring of TCDD-treated dams; it was undetectable in 120-day-old offspring of untreated or TCDD-treated dams (Fig. 4B).

4. Discussion

The results of the present study extend to the lung our previous observation in the liver [27] that gestational and lactational TCDD exposure causes long-lasting induction of CYP1A1 in the offspring of rats. Although the induction of CYP1A1 in the lungs was sustained through young adulthood (postnatal day 40), it was not permanent, nor was its induction in the liver in our earlier study [27]. The lack of

permanence of the induction is evidenced by the similarity in the level of EROD activity in 120-day-old offspring of control and TCDD-treated mothers. Given the biological half-life (26–39 days) of TCDD in the rat [43], tissue levels resulting from the administered doses of the compound might have been sufficient to effect its induction only through young adulthood (i.e. postnatal day 40), but not in 120-day-old offspring. Studies of the relationship between tissue TCDD levels and CYP1A1 inducibility in the offspring will be necessary to confirm this speculation.

A comparison of CYP1A1 induction, in response to TCDD, in the lungs (our current study) with that in the liver (our previous study [27]) suggests that its induction may be more pronounced or more sustained in the lung than in the liver. This speculation is supported by the reported higher sensitivity of the rat lung than liver to CYP1A1 inducibility [32,38]. Differential tissue levels of TCDD are unlikely to have contributed significantly to the differential tissue induction of the enzyme because the level of the compound in the lungs is reported to be insignificant compared with that in the liver of TCDD-treated rats [44].

The magnitude of induction of CYP1A1 protein was higher than that of EROD activity in lung microsomes from 40-day-old male and female TCDD-exposed rats by a factor of 37 and 13, respectively. We observed a similar, but less pronounced, disparity between the level of the protein and its activity in liver microsomes from 25-day-old male rats in our previous study [27]. The cause of the disparity in the current study was not determined. However, lipid peroxidation, which yields catalytically nonfunctional cytochromes P450 [30,45,46], and is enhanced in TCDD-treated animals [47,48], was a likely contributing factor. Evidence from our previous study [27] had suggested that enhanced lipid peroxidation probably contributed to the disparity in liver microsomes.

Among the major findings in the current study was the induction of CYP1A2 in rat lungs. Our observed induction of the enzyme is in agreement with the reports of some investigators that the enzyme is inducible in the lungs of rodents and other species [23,24] but contrasts with the reported lack of induction of the enzyme in the lungs of TCDD-treated adult rats [44]. Several factors, including differences in the age of the animals at the time of TCDD exposure, could contribute to the disparate observations. We did not determine the level or activity of the enzyme in offspring of TCDD-exposed dams younger than 40 days of age and, hence, do not know the age of onset of the induction. However, based on the reported low expression and inducibility of CYP1A2 in the immature compared with adults of many mammalian species [49–51], the induction could be assumed to have occurred largely after the neonatal period.

It should be pointed out that the reports of the resistance of neonates to CYP1A2 expression and inducibility [49–51] are based on studies in the liver and may not necessarily apply to extrahepatic tissues. Tissue-specific regulatory factors have been implicated in the tissue-specific expression of CYP1A2 [52]. Perinatal levels of these factors may be constitutively higher or more inducible by TCDD in the lungs than in the liver, and this pattern may be different in adulthood. These speculations are supported by our observation that CYP1A2 was induced in parallel with CYP1A1 in the lungs (in the current study) but not in the liver (in our previous study [27]). The observation is in agreement with the reported coordinate [1–3,14] as well as independent [15–18] induction of the two enzymes. Another notable observation in the current study was the higher induction of pulmonary CYP1A2 in female than in male rats. Other studies have reported higher inducibility of hepatic CYP1A in female than in male rats [53–57]. However, only a few of these studies [53,54] have examined gender differences in the induction in extrahepatic tissues. Studies comparing immature and adult rats as well as male and female rats in terms of (i) tissue levels of CYP1A regulatory factors, and (ii) the time courses of CYP1A2 induction in hepatic and extrahepatic tissues would enhance our understanding of the tissue-, age- and gender-specificities in the induction of CYP1A2 expression. The studies would enhance our understanding of the mechanisms of the coordinate and independent regulation of expression of CYP1A1 and CYP1A2 as well.

In humans, CYP1A2 is generally considered to be predominantly hepatic and to be absent in the lungs [58–60]. However, some investigators have reported the presence of the enzyme and its transcript in lung tissues [61] and others have detected transcripts of the enzyme in esophageal tissues [62]. The relevance to humans of our observed inducibility of the enzyme in rat lungs also remains to be determined.

Acknowledgments

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