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Effect of Gestational and Lactational 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin Exposure on the Level and Catalytic Activities of Hepatic Microsomal CYP1A in Prepubertal and Adult Rats

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ABSTRACT. We determined the inducibility, as well as the persistence of the induction, of hepatic microsomal CYP1A1 and CYP1A2 (by western blot analysis), and their catalytic activities (as measured by resorufin ether *O*-dealkylation) in prepubertal (25-day-old) and adult (120-day-old) offspring of timed-pregnant Sprague–Dawley rats treated with 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). TCDD treatment was subcutaneous, at a low dose of 0.1 μg/kg, on gestational days 7, 14, and 20, and on lactational days 7 and 14. CYP1A1 protein was induced significantly (23-fold) in prepubertal but not in adult offspring of TCDD-exposed dams, whereas ethoxyresorufin *O*-deethylase (EROD) activity, which is CYP1A1-preferential, was induced less extensively (5-fold) and slightly (1.7-fold) in the prepubertal and adult offspring, respectively. Benzyloxyresorufin *O*-debenzylase (BROD) activity, which is CYP2B-preferential but has been reported to be catalyzed by CYP1A1, was also induced 5- and 6-fold in prepubertal and adult offspring, respectively, of TCDD-exposed dams. However, the induced BROD activity was neither inhibited by antibody against CYP1A1 nor accompanied by an elevated level of microsomal CYP2B. CYP1A2 was induced slightly only in prepubertal offspring of TCDD-treated dams. There was suggestive evidence of enhanced lipid peroxidation in hepatic microsomes from prepubertal but not adult offspring of TCDD-treated dams. These data showed that *in utero* plus lactational TCDD exposure effected transient induction of hepatic microsomal CYP1A1 but sustained induction of BROD activity, which may be catalyzed by enzymes other than CYP1A or CYP2B. BIOCHEM PHARMACOL **59**;9: 1147–1154, 2000. © 2000 Elsevier Science Inc.

KEY WORDS. CYP1A; CYP2B; EROD; BROD; *in utero*; lactational TCDD exposure

CYP1A1 induction is among the most potent and best characterized of the effects of TCDD¶ [1, 2]. TCDD is also among the most toxic chemicals known, with the toxic effects including teratogenicity and other developmental defects [3–5], many of which may be enhanced by CYP1A1 induction by the compound [6–8]. CYP1A1 is the enzyme associated most closely with AHH activity [9], which catalyzes the bioactivation of many procarcinogenic and promutagenic polyaromatic hydrocarbons (e.g. benzo[*a*]pyrene) to their ultimate reactive intermediates [10]. CYP1A1 induction by TCDD is mediated by the ligand-activated transcription factor AhR. Following TCDD binding, the resulting receptor complex translocates to the nucleus and binds to a nuclear transcription factor, ARNT [1, 7]. The AhR–ARNT complex then binds to consensus sequences in the regulatory domains of the *CYP1A1* gene [1, 6, 7]. Additional target genes of the AHR–ARNT complex include those encoding other enzymes of endogenous and foreign compound metabolism, including CYP1A2 [6, 7] and CYP1B1 [7]. Elevated levels of the enzyme in the immature organism are of toxicological interest, partly because of the potential for enhanced susceptibility to the teratogenic and carcinogenic effects of chemicals.

In utero and lactational exposure to TCDD has been reported in the fetus and neonate of experimental animals [11, 12] and humans [13], with the exposure in experimental animals reported to cause induction of AHH activity [14, 15]. In one of these studies [14], administration of a

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¶ *Abbreviations*: AHH, aryl hydrocarbon hydroxylase; AhR, aryl hydrocarbon receptor; ARNT, AhR nuclear translocator; AUC, area under the curve; BROD, benzyloxyresorufin *O*-debenzylase; EROD, ethoxyresorufin *O*-deethylase; LAHP, linoleic acid hydroperoxide; MROD, methoxyresorufin *O*-demethylase; PAH, polyaromatic hydrocarbon; TBARS, thiobarbituric acid-reactive substances; and TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin.

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single 2.5 μg/kg i.p. dose of TCDD to pregnant rats caused a 100-fold induction of fetal hepatic AHH activity, as well as extensive damage to the liver and other tissues in the dam and fetus. In another study [15], administration of a single 3 μg/kg oral dose of the compound to pregnant rats caused an 18.5-fold induction of hepatic AHH activity in 1-day-old offspring. In the latter study [15], the activity remained induced through postnatal day 21, at which time the activity was 3- to 5-fold that of the controls. The induction in the latter study was attributed predominantly to lactational (rather than transplacental) transfer of the compound to the offspring. In neither of the two studies [14, 15], however, was CYP1A1 protein determined.

TCDD has a long biological half-life (26–39 days) in the rat (Ref. 11 and references therein), a factor that accounts for some of the sustained effects of the compound [7]. The current study was conducted to determine the extent of hepatic CYP1A induction in offspring of pregnant and lactating rats treated repeatedly with a low, nontoxic dose (0.1 μg/kg) of the compound, and to assess the degree of persistence of the induction. Our results show that the exposure caused transient induction of CYP1A1 and its catalytic activity but with the magnitude of induction of the protein being much greater than that of its catalytic activity. BROD activity, which, although CYP2B-preferential [16, 17], has been speculated to be catalyzed by CYP1A [17–19], was induced by TCDD, with the induction being more sustained than that of EROD activity. However, neither CYP1A nor CYP2B appeared to be responsible for the induced BROD activity.

MATERIALS AND METHODS

Materials

TCDD was obtained from Cambridge Isotopes; NADPH, $NADP^+$, 2-thiobarbituric acid, 1,1,3,3-tetraethoxypropane (malondialdehyde), linoleic acid, and soybean lipoxygenase were obtained from the Sigma Chemical Co. The following were obtained from the commercial sources indicated in parentheses: ethoxyresorufin (Pierce Chemical Co.); glucose-6-phosphate dehydrogenase (Boehringer-Mannheim); methoxyresorufin, benzyloxyresorufin, and resorufin sodium (Molecular Probes); and ECL western blotting analysis system (Amersham Life Science). LAHP was prepared as described previously [20]. CD5, a mouse monoclonal antibody against rat liver CYP1A1 that cross-reacts with CYP1A2 [21], and BEA, a mouse monoclonal antibody against rat liver CYP2B2 that cross-reacts with CYP2B1 and CYP2A1 [22], were prepared as described in the references indicated. Antibody against recombinant CYP1B1 was provided by Dr. Colin Jefcoate.

Animals and Pretreatment

Twelve timed-pregnant Sprague–Dawley rats (Taconic Farms), weighing 200–230 g, were housed individually in polypropylene shoebox cages (43 × 22 × 20 cm) with wood shavings for bedding and free access to food and water. Colony room lights were turned on automatically at 8:00 a.m. and off at 8:00 p.m. Four dams were injected s.c. 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 *post partum* days 7 and 14, for a cumulative dose of 0.5 μg/kg. Four dams were treated with corn oil only, and another four were untreated. The corn oil used was free of peroxides as described previously [23]. Each liter was culled to a maximum of 8 pups at birth. Body weight growth curves were recorded throughout the duration of the study, and liver-to-body weight ratios were determined at euthanasia. Offspring euthanized for study at 25 days of age are referred to interchangeably as weanlings or prepubertal rats; offspring euthanized for study at 120 days are termed adults.

Preparation of Microsomes

On postnatal day 25 or 120, male offspring were euthanized; their livers were isolated and frozen immediately in liquid nitrogen, and then stored at −80° for the isolation of washed, hemoglobin-minimized microsomes by differential centrifugation as described previously [24].

Electrophoresis and Western Blot Analysis

Western blot analysis of microsomes was performed following SDS–PAGE as described previously [25]. CYP1A1 and CYP1A2 were detected with CD5 [21], and CY2B1 and CYP2B2 were detected with BEA [22]. CYP1B1 was probed with a mouse antibody against recombinant CYP1B1 (provided by Dr. Colin Jefcoate of the University of Wisconsin). Autoradiographic bands on the blots were detected with a horseradish peroxidase-conjugated secondary antibody by enhanced chemiluminescence according to the manufacturer's instructions. Bands on the exposed films were quantified by densitometry using a Bio Image IQ scanner equipped with a data station. Each densitometric (AUC) value was linear with respect to the amount of protein analyzed.

Alkoxyresorufin O-Dealkylase Assays

Microsomal *O*-dealkylation of ethoxyresorufin or methoxyresorufin as a preferential activity of CYP1A1 or CYP1A2, respectively [16, 17, 26], or benzyloxyresorufin as a potential alternative activity of CYP1A1 [17–19], was assayed fluorometrically as described by Pohl and Fouts [27], at a substrate and protein concentration of 5 μM and 50 μg/mL, respectively, with minor modifications as specified in the legends to figures and tables.

Microsomal Lipid Peroxidation

Non-enzymatic microsomal lipid peroxidation was assayed as an indirect measure of the level of preformed oxidized lipids in microsomes [28], using the colorimetric thiobarbi-

turic acid procedure as described previously [20, 28], and LAHP instead of NADPH to initiate the reaction. We used LAHP because all of our microsomal preparations contained EDTA (100 μM), which is inhibitory to NADPH-dependent but not LAHP-dependent microsomal lipid peroxidation [20]. The added LAHP contributes minimally to total TBARS formation in the assay [20]. Furthermore, the magnitude of hydroperoxide-induced TBARS formation in microsomes parallels the levels of endogenous polyunsaturated fatty acid peroxides and related oxidized lipid species preformed in the endoplasmic reticulum, and is related inversely to the antioxidant content of the microsomal preparation [28, 29].

Other Assays

Total microsomal cytochrome P450 content was determined spectrophotometrically [30]. Protein was determined by the method of Lowry *et al.* [31].

Statistical Analysis

Data are presented as means ± SD. Differences between means of multiple groups were analyzed statistically, using Duncan's multiple range test ($P < 0.05$) as described by Gad and Weil [32]. In experiments in which Bartlett's test for homogeneity of variance showed heterogeneity between groups, two-tailed Student's or Cochran's *t*-test was used for pairwise comparisons, with the level of significance determined at $P < 0.05$.

RESULTS

Treatment with corn oil alone or TCDD did not affect body weights of the dams in comparison with untreated control dams. 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.

Effect of TCDD Exposure on Hepatic Microsomal EROD or MROD Activity

In offspring of untreated dams, EROD but not MROD activity was slightly but significantly ($P < 0.05$) higher in weanlings (85.3 ± 4.0) than in adults (64.5 ± 10.0) (Table 1). Similarly, in offspring of corn oil only-exposed dams, EROD but not MROD activity was significantly higher ($P < 0.05$) in weanlings (162.2 ± 39.0) than in adults (66.7 ± 14.0) (Table 1).

In offspring of TCDD-treated dams, EROD activity was induced 5- and 1.7-fold in prepubertal and adult rats, respectively, relative to the activity in their corresponding corn oil only-treated counterparts (Table 1). MROD activity was also induced in prepubertal and adult offspring of TCDD-treated mothers, but the induction, in contrast to that of EROD activity, was comparable in weanlings (1.9-fold) and adults (1.7-fold), and did not reach the level of statistical significance (Table 1).

TABLE 1. Alkoxyresorufin O-dealkylase activities in liver microsomes from offspring of control and TCDD-treated dams

Maternal treatment	Resorufin ether O-dealkylase activities (pmol resorufin/mg/min)	
	Postnatal age (days)	
	25	120
	EROD activity	
None	85.3 ± 4.0[a]	64.5 ± 10.0[a]
Corn oil	162.2 ± 39.0[b]*	66.7 ± 14.0[ab]
TCDD	815.3 ± 204[c]*	119.0 ± 33.0[b]
	MROD activity	
None	119.0 ± 13.0	104.0 ± 16.0
Corn oil	202.0 ± 83.0	146.0 ± 25.0[ab]
TCDD	384.0 ± 199.0	250.0 ± 29.0[b]
	BROD activity	
None	23.0 ± 10.0[a]	17.0 ± 4.0[a]
Corn oil	26.0 ± 6.2[a]*	8.3 ± 4.0[b]
TCDD	129.0 ± 13.0[b]*	48.0 ± 7.2[c]

Each determination, in triplicate 0.5-mL incubations, contained 25 μg of microsomal protein from offspring of untreated, corn oil-treated, or TCDD-treated rats, and 5 μM ethoxyresorufin, methoxyresorufin, or benzyloxyresorufin. The assay, at 37° for 10 min, was initiated by the addition of an NADPH-generating system [22] to two of the three incubations, with the third serving as an NADPH blank. Each value is the mean (± SD) of determinations in 3–5 rats.

[a,b,c]Values within the same column not bearing identical superscripts are significantly different from each other ($P < 0.05$).

*Significantly different from similarly treated 120-day-old offspring ($P < 0.05$).

Effect of TCDD Exposure on Total Hepatic Microsomal Cytochrome P450 Levels

The content of total, spectrally measurable cytochrome P450 (nmol/mg microsomal protein) was 0.38 ± 0.13, 0.40 ± 0.17, and 0.44 ± 0.07 in prepubertal offspring of untreated, corn oil only-treated, and TCDD-treated mothers, respectively, and 0.70 ± 0.11, 0.65 ± 0.03, and 0.70 ± 0.13 in adult offspring of untreated, corn oil only-treated, and TCDD-treated mothers, respectively (data not shown). Thus, *in utero* and lactational exposure to corn oil or TCDD did not affect the total P450 content in the prepubertal or adult offspring, and did not alter the characteristically higher total content of the hemoprotein in the adult than in the immature animal [24].

Western blot analysis of microsomal cytochrome P450 is shown in Fig. 1, and quantification of the western blot data is shown in Fig. 2. In prepubertal offspring of untreated dams, CYP1A1 protein was present at a very low but quantifiable level (Fig. 2). The level of the enzyme protein was increased 2.4-fold in prepubertal offspring of corn oil only-treated dams relative to their untreated counterparts, and 23-fold in prepubertal offspring of TCDD-exposed mothers relative to their corn oil only-treated counterparts (Fig. 2). In adult rats, in contrast, CYP1A1 was undetectable in offspring of untreated, corn oil only-treated, or TCDD-treated dams (Fig. 2). The TCDD-mediated

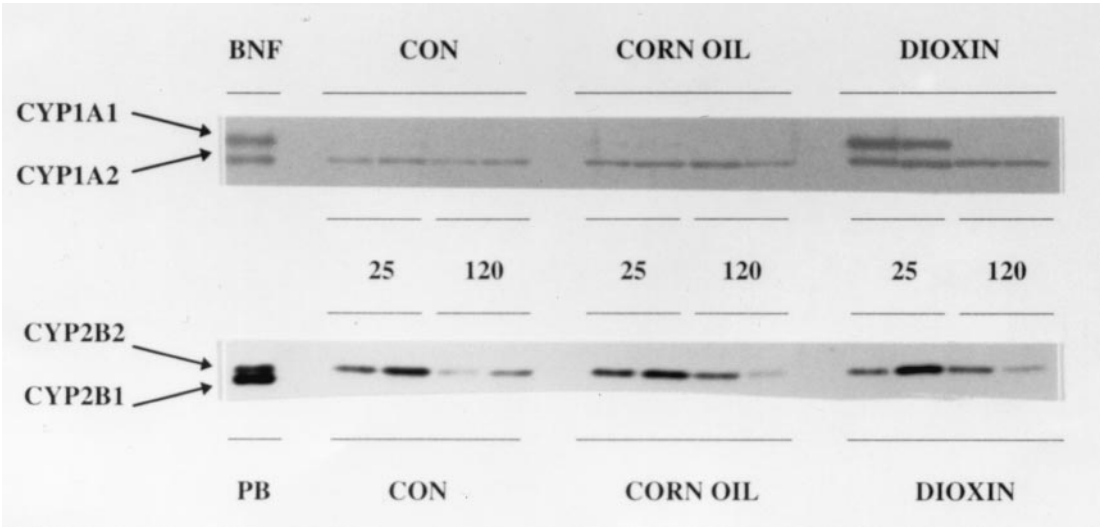

FIG. 1. Western blot analysis of liver microsomal CYP1A and 2B. Electrophoresed liver microsomal proteins were probed with an antibody that recognizes either CYP1A1 and CYP1A2 (CD5) or CYP2A1, CYP2B1, and CYP2B2 (BEA). Numbers (25, 120) between the CYP1A1/CYP1A2 and CYP2B1/CYP2B2 panels represent ages of the animals in days. Each lane represents pooled microsomes from the livers of two rats. Amounts of liver microsomal protein analyzed were: 0.5 μg for BNF (microsomes from β-naphthoflavone-treated rats as a positive control for CYP1A1/2), 1.25 μg for PB (microsomes from phenobarbital-treated rats as a positive control for CYP2B1/2), or 10 μg for microsomes from untreated (CON), corn oil-treated, or TCDD- (DIOXIN) treated rats.

changes in CYP1A1 levels generally paralleled changes in EROD activity, except in prepubertal offspring of TCDD-treated mothers, in which induction of the activity (5-fold) was disproportionately lower than induction of the protein (23-fold) (compare Fig. 2 and Table 1). We observed no significant effect of age or maternal treatment with corn oil on the level of microsomal CYP1A2 in the offspring, whereas maternal TCDD treatment effected a 90% increase in the level of the protein in the prepubertal but not adult offspring (Fig. 2).

Effect of TCDD Exposure on Microsomal BROD Activity

The higher induction of CYP1A1 protein than of its preferential activity, EROD, observed above prompted us to examine whether another CYP1A1 activity paralleled the induction of the protein. We chose BROD, which, although CYP2B-preferential [16, 17], has been speculated to be catalyzed by CYP1A [17–19]. Table 1 shows that in prepubertal offspring of TCDD-exposed dams, BROD ac-

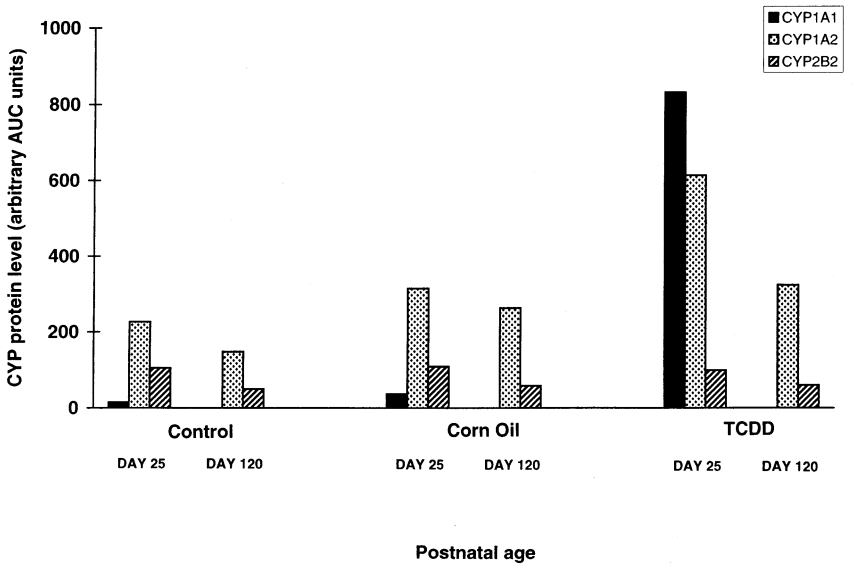

FIG. 2. CYP protein levels in offspring of untreated, corn oil-treated, or TCDD-treated dams. Values are based on densitometric analysis of the western blots shown in Fig. 1. Each value represents the average densitometric determinations from the paired lanes shown in Fig. 1.

TABLE 2. Effect of antibody to either CYPIAI (MAb C7) or MAb C8) or both CYPIAI and CYPIA2 [anti-P450d(+c)] on BROD activity in microsomes from 25- or 120-day-old offspring of TCDD-treated dams

Antibody or IgG added	BROD activity (pmol resorufin formed/mg/min)	
	Postnatal age (days)	
	25	120
None	141.5	51.3
MAb C7	143.1 (100)	52.6 (100)
MAb C8	136.2 (95)*	47.0 (89)*
IgG	163.4 (100)	54.3 (100)
Anti-P450d(+c)	148.7 (91)†	42.8 (78)†

The assay was described in the legend to Table 1 except that incubation mixtures also contained either 6.25 μg MAb C7, 6.25 μg MAb C8, 0.15 mg rabbit IgG, or 0.15 mg anti-P450d(+c), and benzyloxyresorufin (5 μM) only, and were preincubated at 37° for 5 min at room temperature prior to initiating the reaction. Each value is the average of two determinations in microsomes from 2 animals, each in duplicate, from which an NADPH blank value has been subtracted.

*†Values in parentheses are percentages of MAb C7 (*) or IgG (†) controls.

tivity was induced significantly ($P < 0.05$), and that the induction (5.2-fold) was comparable to that of EROD activity. The activity was also induced significantly (5.8-fold) in adult offspring of TCDD-treated dams when compared with the activity in their corn oil only-treated counterparts (Table 1). It should be noted that the activity was lower in adult offspring of corn oil only-treated dams, when compared with their untreated counterparts. However, a statistically significant induction of the activity (2.8-fold) was still observed in adults when the activity in TCDD-treated offspring was compared with that in offspring of the untreated dams (Table 1). We have no explanation for the selective depression of BROD activity in adult offspring of corn oil only-treated dams. The induction of EROD activity in prepubertal offspring of TCDD-exposed dams, in which CYP1A1 was induced, as well as in adult offspring of TCDD-treated dams, in which CYP1A1 was not induced, suggested that the observed TCDD-induced BROD activity was not catalyzed by CYP1A1.

Effect of Antibodies to CYP1A1 and CYP1A2 on Microsomal BROD Activity

We further assessed the role of CYP1A in the induced BROD activity in microsomes from prepubertal and adult offspring of TCDD-treated dams by determining the effect of antibodies against CYP1A1 and CYP1A2 on the activity. Table 2 shows that BROD activity was not inhibited significantly by an antibody that recognizes either CYP1A1 alone or both CYP1A1 and CYP1A2, strongly indicating that neither CYP1A1 nor CYP1A2 was involved in the induced BROD activity. EROD activity, in contrast, was inhibited 92 and 79% by C8 (inhibitory mouse monoclonal antibody to rat liver CYP1A1) and anti-P450d(+c) (rabbit polyclonal antibody to rat liver CYP1A2 that cross-reacts with CYP1A1), respectively (data not shown).

Effect of TCDD Exposure on Microsomal CYP2B and 1B1 Protein Levels

Because BROD is a preferred activity of CYP2B [16, 17], we assessed whether the TCDD-induced BROD activity resulted from induction of CYP2B by TCDD. Figure 2 shows the profile of hepatic CYP2B in offspring of untreated, corn oil-treated, or TCDD-treated dams. Figure 2 also shows that CYP2B2, but not CYP2B1, was detectable in prepubertal or adult offspring of untreated rats, with the abundance of the enzyme being higher in prepubertals than in adults. As also shown in Fig. 2, maternal treatment with either corn oil alone or TCDD had no effect on the level of the enzyme in the prepubertal or adult offspring (Fig. 2). Thus, the elevated BROD activity observed in prepubertal or adult offspring of TCDD-treated dams was also unlikely to be catalyzed by CYP2B. Further assessment of the extent of CYP2B participation in the reaction was precluded by the unavailability to us of an inhibitory antibody that is CYP2B2-specific.

CYP1B1 is also inducible by PAHs, including TCDD [7, 33]. The enzyme could have been induced and, conceivably, could have contributed to the induced BROD activity in the current study and in previous reports [17–19]. However, we did not detect CYP1B1 protein in any of the microsomal preparations in the current study (data not shown).

Effect of TCDD Exposure on LAHP-Stimulated Microsomal Lipid Peroxidation

Possible factors that could have contributed to our observation of more than 4-fold higher induction of CYP1A1 protein than of its catalytic activity include loss of the enzyme heme as a result of oxidative stress and lipid peroxidation [34]. This effect has been observed in the rat with a few inducers of CYP1A1, including 3,3′-dichlorobenzidine [20, 28, 35] and pyridine [25]. TCDD has also been reported to stimulate oxidative stress in animals [36, 37], with concomitant diminution of CYP1A1 activity [19]. Therefore, we initiated experiments aimed at assessing the potential of *in utero* and lactational TCDD exposure to stimulate oxidative stress in the rat. As shown in Fig. 3, only in the prepubertal offspring was there evidence of enhanced lipid peroxidation by TCDD treatment.

DISCUSSION

We utilized CYP1A induction, one of the most sensitive effects of TCDD [1, 6–8], as a marker of the effect of the compound in offspring of TCDD-exposed dams. Our results show that CYP1A1 induction, as well as EROD activity, was induced in offspring of TCDD-exposed mothers but that the induction was transient, being pronounced at 25 days but not at 120 days after birth. We did not determine the induction in the offspring at an age earlier than 25 days. It is highly likely that the induction was more pronounced

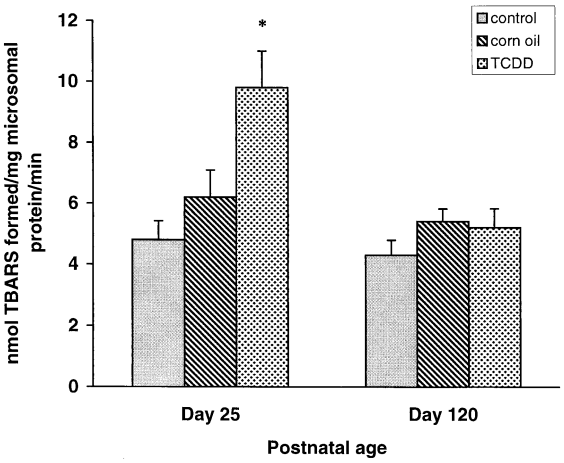

FIG. 3. Lipid peroxidation in liver microsomes from offspring of control, corn oil-treated, and TCDD-treated rats. For each determination, microsomes (1 mg protein/mL of 0.1 M phosphate buffer, pH 7.4, containing 100 μM EDTA) were incubated in triplicate at room temperature, and lipid peroxidation was initiated by the addition of 250 μM LAHP to two of the three incubations. The assay was terminated after a 2.5-min incubation, and TBARS were determined colorimetrically [29]. Each value is the mean (± SD) of determinations in microsomes from 3 rats. Key: (*) significantly different from 25-day-old offspring of untreated or corn oil-treated dams or from 120-day-old offspring of TCDD-treated dams ($P < 0.05$).

at an earlier age, when the tissue burden of the compound may have been higher, than the 5-fold induction observed in the 25-day-old animals. A 100-fold induction of hepatic AHH activity in fetuses of pregnant rats treated with a single 2.5 μg/kg i.p. dose of TCDD on gestational day 17 has been reported previously [14]. In another, earlier study [15], administration of a single 3 μg/kg oral dose of the compound to pregnant rats on gestational day 16 caused an 11-fold induction of hepatic AHH activity in the fetus. The postnatal induction was maximal (18.5-fold) 1 day after birth, but lower (3-fold) in 8-day-old offspring [15]. However, CYP1A1 protein was not determined in either of the two earlier studies.

We observed a 23-fold induction of CYP1A1 protein in contrast with only a 5-fold induction of its catalytic activity in prepubertal offspring of TCDD-treated dams. We did not establish the basis of this disparity between immunodetectable protein levels and catalytic activity of the enzyme. However, our observed suggestive enhancement of lipid peroxidation in the microsomes strongly argues for the involvement of TCDD-stimulated peroxidative activities in the decreased CYP1A1 activity. A similar effect has been observed with other CYP1A1 inducers [23, 25]. Furthermore, TCDD has been reported to stimulate oxidative stress, with concomitant diminution of CYP1A1 activity [37]. Nevertheless, assessment of the direct involvement of enhancement of lipid peroxidation in the diminution of CYP1A1 activity in TCDD-exposed preweanlings will require further studies.

AHH induction in neonates of TCDD-treated dams in an earlier study was attributed predominantly to lactational rather than to transplacental transfer of the compound to the offspring [15]. However, the reported 100-fold induction of fetal hepatic AHH activity following maternal TCDD treatment [14] indicates transplacental transfer of the compound. If our observed CYP induction in prepubertals resulted predominantly from lactational exposure to TCDD, the induction would have to be attributed predominantly to the last two 0.1 μg/kg doses of the compound that we administered to the lactating dams. The induction would also underscore the potency of TCDD in the immature organism, as the tissue levels resulting from the exposure are likely to have been very low. We did not determine the tissue levels of TCDD and, hence, do not know the tissue levels of the compound associated with the induction we observed. However, the levels should be much lower than those resulting from the 3 μg/kg [15] or 2.5 μg/kg [14] doses administered in previous studies, in which tissue levels of the compound were also not determined.

Interestingly, CYP1A2 was not induced in parallel with CYP1A1 in the present study. This observation is consonant with the reported lower inducibility of CYP1A2 than CYP1A1 in the female rat by TCDD [33], but contrasts with the reported higher inducibility of CYP1A2 than CYP1A1 in the adult male mouse [38, 39]. The basis of the differential induction by TCDD of CYP1A2 in the mouse and rat, given the reported coordinate regulation of the expression of CYP1A1 and CYP1A2 [1, 6, 7], is unknown. Nevertheless, independent regulation of expression of the two enzymes by certain chemicals has been reported [40, 41], and may be more pronounced in the immature organism than in the adult.

We are currently investigating several characteristics of *in utero*/lactational CYP induction by TCDD. These include the role of oxidative stress in the diminished catalytic activity of CYP1A1, and the possible induction or repression of forms of cytochrome P450 other than those examined in the present study. Of relevance to the possible alteration of other forms of CYP by *in utero*/lactational TCDD exposure is our observed induction of BROD activity in the prepubertal as well as adult offspring of TCDD-treated dams. We examined BROD activity in the current study as an additional marker of CYP1A activity because the enzymes have been reported to contribute to BROD activity [17–19], even though the reaction is CYP2B-preferential [17]. Our results strongly indicate that CYP2B did not contribute significantly to the induced BROD activity because the enzyme was not induced in prepubertal and adult offspring of TCDD-treated dams, in which the activity was induced 5- and 6-fold, respectively. The induced BROD activity is equally unlikely to have been catalyzed by CYP1A, as evidenced by the failure of a CYP1A1- or CYP1A1/2-specific antibody to inhibit the reaction. Furthermore, the reaction was induced in adult offspring of TCDD-exposed dams, in which neither CYP1A1 nor CYP1A2 was induced. We speculate that

enzymes other than CYP1A, which are inducible by typical inducers of CYP1A, including TCDD, must be responsible for the induced BROD activity in the present study and in others in which CYP1A involvement in the reaction has been suggested [17–19]. While the identity of the enzyme(s) responsible for catalyzing the induced BROD activity remains to be determined, it can be concluded from our present findings that its induction by perinatal TCDD exposure in the rat may be sustained if not permanent.

There are many potential biological consequences of elevated levels of CYP1A in the immature organism. These include heightened sensitivity to many procarcinogens and promutagens, the effects of which require bioactivation by CYP1A. In addition, CYP1A1 and CYP1A2 catalyze the biotransformation of endogenous estrogens [42] and androgens [43]. The consequences would be expected to include altered critical levels of the hormones essential for normal developmental functions. Altered levels of these hormones could account for many of the teratogenic consequences, including biochemical and behavioral demasculinization [5] in male offspring of TCDD-treated rats. Whether these effects are attainable at the doses of TCDD used in the current study remains to be determined.

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