

A Maternal *Ahr* Null Genotype Sensitizes Embryos to Chemical Teratogenesis*

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Tami L. Thomae, Edward Glover, and Christopher A. Bradfield‡

From the McArdle Laboratory for Cancer Research, University of Wisconsin Medical School, Madison, Wisconsin 53706

The aryl hydrocarbon receptor (encoded by the *Ahr* locus) is a ligand-activated transcription factor that mediates the toxicology and teratology of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (dioxin). In an effort to understand the role of the maternal compartment in dioxin teratology, we designed a breeding strategy that allowed us to compare the teratogenic response in embryos from *Ahr*^{-/-} (null) and *Ahr*^{+/+} (wild-type) dams. Using this strategy, we demonstrate that embryos from the *Ahr*^{-/-} dams are 5-fold more sensitive to dioxin-induced cleft palate and hydronephrosis as compared with embryos from an *Ahr*^{+/+} dam. Moreover, this increased teratogenic sensitivity extends beyond dioxin, because embryos from *Ahr*^{-/-} dams exhibited a 9-fold increase in their sensitivity to the fetotoxic effects of the glucocorticoid, dexamethasone. In searching for an explanation for this increased sensitivity, we found that more dioxin and dexamethasone reached the embryos from *Ahr*^{-/-} dams as compared with embryos from *Ahr*^{+/+} dams. We propose that increased deposition of teratogens/fetotoxicants to the embryonic compartment is the result of porto-systemic shunting and/or blocked P4501A induction in *Ahr*^{-/-} dams. In addition to demonstrating the importance of maternal AHR in teratogenesis, these data may have implications that reach beyond the mechanism of action of dioxin. In this regard, the *Ahr*^{-/-} mouse may provide a system that allows pharmacological agents and toxicants to be more easily studied in a model where first pass clearance is a significant obstacle.

Chemical teratogens can perturb normal mammalian development through direct action on the embryo, indirectly through maternal toxicity, or as the result of a combination of both of these mechanisms. The maternal compartment can afford protection by functioning as a metabolic or physical barrier that reduces embryonic exposure to a given agent (1). The maternal compartment also holds the potential to serve as a site of teratogen bioactivation and may serve to increase the concentrations of active metabolites that directly perturb normal embryonic development (2, 3). Understanding the relative contributions of maternal and embryonic physiology for a given teratogen can be difficult, especially when chemical agents induce a variety of teratogenic end points or display remarkable pharmacological potency.

One approach to understanding the relative contributions of the maternal and embryonic physiology is to define these two compartments via their respective genotypes. Such an ap-

proach is particularly powerful when working with null alleles that have been generated via gene targeting. In such cases, the dominance order is clear, as is the functional relationship between one or two copies of the wild-type allele. Through appropriate genetic crosses one can then generate informative combinations of maternal and embryonic genotypes that can help identify when maternally or embryonically expressed loci play a differential role in the teratogenic response to a specific chemical (4).

Based on these ideas, we began a series of experiments to determine the role that the maternal compartment plays in the teratogenicity of the environmental contaminant, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (dioxin).¹ In mice, the hallmark teratogenic responses to dioxin are clefting of the palate and hydronephrosis (5–9). Previous reports have provided compelling evidence that dioxin-induced cleft palate is likely to be a direct response of this compound on the developing embryo (9). Given that teratogenic doses of dioxin, 25 µg/kg, are near those that are likely to induce various aspects of toxicity in the dam (e.g. thymic atrophy and metabolizing enzyme induction), we became interested in determining whether maternal toxicity was playing any role in dioxin teratology (10, 11).

We attempted to take advantage of the fact that essentially all aspects of dioxin action require signal transduction through a ligand-activated transcription factor known as the aryl hydrocarbon receptor (AHR) (12–21). Given that mice harboring null alleles of the *Ahr* locus are viable, we were able to generate cohorts of animals where the maternal genotype was homozygous for either the wild-type (*Ahr*^{+/+}) or null allele (*Ahr*^{-/-}). Through crosses to the appropriate paternal genotype, all embryos generated were heterozygous for the wild-type and null alleles. With embryo genotype identical, we could then attribute any differential response/sensitivity to the differences in the maternal genotypes at *Ahr*.

Our initial hypothesis was that *Ahr*^{+/+} dams would give rise to embryos that were more sensitive to dioxin-induced terata. This was based on the idea that AHR-mediated toxicity within the maternal compartment would exacerbate the teratogenic action of dioxin in the developing embryos. What we observed was the opposite. We found that embryos from *Ahr*^{-/-} dams were five times more sensitive to the teratogenic action of dioxin. To explain this observation, we demonstrate that dams lacking a functional AHR provide a less efficient maternal barrier and allow increased concentrations of chemical teratogens to reach the developing embryo. Our additional observation that this decreased maternal barrier extends to dexamethasone-induced fetotoxicity suggests that this system may provide a general model for the more sensitive detection of chemical teratogens.

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‡ To whom correspondence should be addressed: McArdle Laboratory for Cancer Research, 1400 University Ave., Madison, WI 53706-1599. Tel.: 608-262-2024; Fax: 608-262-2824; E-mail: Bradfield@oncology.wisc.edu.

¹ The abbreviations used are: dioxin, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; AHR, aryl hydrocarbon receptor; *En*, embryonic day *n*; TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; DV, ductus venosus; MOPS, 4-morpholinepropanesulfonic acid.

EXPERIMENTAL PROCEDURES

Mice—The animals were housed in a specific pathogen-free facility on corn cob bedding with food and water *ad libitum* according to the rules and regulations set by the University of Wisconsin. Congenic $Ahr^{-/-}$ mice were generated by 20 backcrosses (N20) to the C57BL6/J background with continual selection for the Ahr null allele (16). All of the $Ahr^{+/+}$ mice were C57BL6/J mice originally obtained from Jackson Laboratories (Bar Harbor, ME) and bred "in house."

Dose Response in Vivo Experiment—Females ($Ahr^{+/+}$ or $Ahr^{-/-}$) were weighed at E0. E0 is defined as the time directly prior to females being mated for 12 h to $Ahr^{-/-}$ or $Ahr^{+/+}$ male mice. At E10, the females were weighed, and those gaining at least two grams were assumed pregnant. Pregnant females were dosed intraperitoneally with 0.03, 0.3, 0.7, 2.0, 5.0, 15.0, 25.0, 48.0, or 64.0 μg of dioxin/kg of body weight. At E18, the embryos and placentae were harvested and weighed. The embryos were scored for sex based on internal reproductive anatomy. Hydronephrosis was scored based on gross examination of the urogenital tract and observation of swelling of the kidneys and ureters with urine. Normal palatogenesis was assessed based on gross examination of the palate surface after an incision was made through the temporal-mandibular joint. Cleft palate was scored if there was not fusion between the secondary palatal shelves. Three millimeters of tail was taken from each embryo for DNA to confirm genotype.

Palate Culture in Vitro Experiment—At E13, the embryos were harvested from the uterus, and the palatal shelves were dissected from embryos. The shelves were separated from the embryo proper by an incision at the temporal-mandibular joint, and the cerebellum and cerebellum were removed by dissection. The remaining opposing shelves were placed into cold $1\times$ phosphate-buffered saline. Suspended palate organ cultures were performed as described (22, 23). The shelves were cultured in 1:1 Dulbecco's modified Eagle's medium/Ham's F-12 medium supplemented with 1% L-glutamine, 1% ascorbate, and 1% penicillin/streptomycin (w/v). The cultures were treated with varying concentrations of dioxin in Me_2SO or with Me_2SO alone. After 72 h, the organ cultures were fixed in 10% neutral buffered formalin and stained with hematoxylin for visualization. Fusion of the opposing palatal shelves was assessed under microscopy and scored as either fused or not fused.

Receptor Ligand Binding Affinity—To measure levels of functional AHR, photo affinity labeling experiments were performed on each of the dams, $Ahr^{-/-}$ and $Ahr^{+/+}$ and the respective $Ahr^{+/-}$ progeny cohorts (i.e. from either $Ahr^{-/-}$ or $Ahr^{+/+}$ dams). At E18, the embryos were harvested, and the livers were dissected from the embryos. The livers from each litter were pooled, and the cytosolic fraction was isolated and used for photo affinity labeling experiments as described previously (24, 25). The photo affinity ligand 2-azido-3- ^{125}I iodo-7,8-dibromodibenzo-*p*-dioxin was added to a final concentration of 1 nM into tubes containing 150 $\mu\text{g}/\text{ml}$ of cytosolic protein. The mixture was incubated at 20 $^{\circ}\text{C}$ for 30 min, followed by a 5-min incubation on ice. Unbound ligand was removed by the addition of a 10% volume of charcoal/gelatin (10%/1%, w/v) in MEN (25 mM MOPS, pH 7.4, 1 mM EDTA, and 0.02% NaN_3) and incubated for 10 min on ice. The mixture was subjected to centrifugation ($2000\times g$, 10 min, 4 $^{\circ}\text{C}$) to remove the charcoal. The supernatant was then irradiated (300 nm at 4 cm) with four Photodyne lamps for 1 min. The sample was then precipitated in ice cold acetone. After an overnight precipitation at -20°C , the protein was pelleted by centrifugation and rinsed with acetone/water (90%/10%, v/v). The pellet was dissolved in SDS sample buffer. One hundred fifty micrograms of protein was loaded onto a 7.5% polyacrylamide gel and subjected to PAGE (24). After the proteins were separated by electrophoresis, the gel was dried and analyzed by autoradiography. The 97-kDa bands were cut out and counted on a Minaxi counter (Packard Instrument Company, Meridian, CT).

Dexamethasone Survival—Pregnant $Ahr^{+/+}$ and $Ahr^{-/-}$ females harboring $Ahr^{+/-}$ embryos were given an intraperitoneal injection of 15 mg/kg of dexamethasone (Sigma-Aldrich) in Me_2SO at E12. The embryos were assessed for survival at E18.5. Dead embryos were identified through their exhibition of severe necrosis, runting, and abnormal morphology. Conversely live embryos were defined as those exhibiting normal growth and morphology.

High Resolution Chromatography/Low Resolution Mass Spectrometry of Embryonic Dioxin Levels—Pregnant female $Ahr^{+/+}$ and $Ahr^{-/-}$ mice were given an intraperitoneal injection of 25 $\mu\text{g}/\text{kg}$ of dioxin in Me_2SO at E14.5. At E15.5, the embryos were harvested from the uterine tract and placed into an amber glass vial. Three embryos (~ 1.5 grams) from three separate litters of each maternal genotype were pooled, and high resolution chromatography/low resolution mass spec-

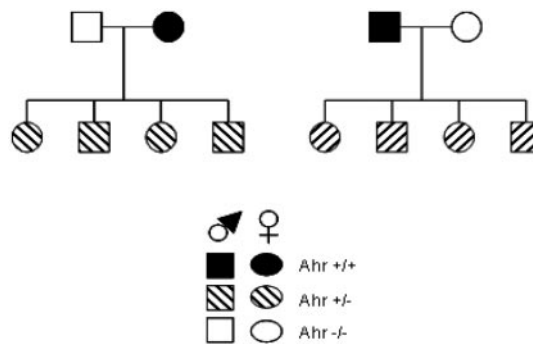


FIG. 1. Breeding strategy to identify role of maternal AHR in dioxin-induced teratogenicity. Solid circles represent $Ahr^{+/+}$ females, and solid squares are $Ahr^{+/+}$ males. Dashed circles and squares represent $Ahr^{+/-}$ females and males, respectively. Open circles and squares represent $Ahr^{-/-}$ females and males, respectively. Lines drawn directly between circles and squares indicate a breeding pair. Lines drawn from a breeding pair to additional circles or squares indicate progeny derived from the specific breeding pair.

trometry was performed to assess the level of the 2,3,7,8-tetrachlorodibenzo-*p*-dioxin isomer present (26). A dioxin standard was provided at a concentration of 625 ng/ml. Analysis was performed by Triangle Laboratories, Inc. (Durham, NC).

Radiometric Measurement of Dioxin Levels—[1,6- ^3H]-tetrachlorodibenzo-*p*-dioxin was purified via high pressure liquid chromatography to a specific activity of 9.02 Ci/mmol (a generous gift from Alan Poland, NCI, National Institutes of Health). Three pregnant female mice of the $Ahr^{+/+}$ and $Ahr^{-/-}$ genotypes were given an intraperitoneal injection at E15 with 15 μCi of [^3H]TCDD. At E16, each individual embryo and each placenta were harvested from the uterine tract. Embryos, placentae, and maternal skin, liver, thymus, kidney, spleen, heart, adipose, intestine, and lung were weighed, and no more than 200 mg of tissue was placed into a scintillation vial containing 1 ml of TS-2 tissue solubilizer (Research Products Inc., Mount Prospect, IL). The tissues were dissolved to completion, and background was minimized with the addition of 100 μl of glacial acetic acid (Fisher). Radioactivity of each sample was measured using the LS6000SC (Beckman-Coulter Inc., Fullerton, CA).

Radiometric Measurement of Dexamethasone Levels—The radiolabeled congener [6,7- ^3H]-dexamethasone was obtained from American Radiolabeled Chemicals, Inc. (St. Louis, MO), at a specific activity of 37 Ci/mmol. Three pregnant mice of the $Ahr^{+/+}$ and two pregnant mice of the $Ahr^{-/-}$ genotypes were given 15 μCi of [^3H]dexamethasone via intraperitoneal injection at E16. Two hours after injection, the embryos and placentae were weighed, and no more than 200 mg of tissue was placed into a scintillation vial containing 1 ml of TS-2 tissue solubilizer (Research Products Inc.). The tissues were dissolved to completion, and background was minimized with the addition of 100 μl of glacial acetic acid (Fisher). The radioactivity of each sample was measured using the LS6000SC (Beckman-Coulter Inc., Fullerton, CA).

RESULTS

Breeding Strategy—The pedigree scheme in Fig. 1 illustrates the breeding strategy used in the subsequent experiments. This scheme allowed for manipulation of the maternal genotypes while keeping the embryonic genotypes the same.

Dose Response, in Vivo—To analyze the susceptibility of embryos to dioxin-induced teratogenesis, we performed dose-response assessment using cleft palate and hydronephrosis as end points. The dams were dosed at E10 with increasing concentrations of dioxin, and the embryos were harvested and scored for teratogenesis at E18. We observed that $Ahr^{+/-}$ embryos derived from $Ahr^{-/-}$ dams were more sensitive to hydronephrosis and cleft palate than $Ahr^{+/-}$ embryos from $Ahr^{+/+}$ dams (Fig. 2). Hydronephrosis in embryos from an $Ahr^{+/+}$ versus and $Ahr^{-/-}$ female exhibited EC_{50} values of 2.5 and 0.5 $\mu\text{g}/\text{kg}$, respectively. Cleft palate in embryos from $Ahr^{+/+}$ versus and $Ahr^{-/-}$ female exhibited EC_{50} values of 48 and 10 $\mu\text{g}/\text{kg}$, respectively.

Dose-response Assessment, Palate Organ Culture—As one method to determine whether the increase in sensitivity of

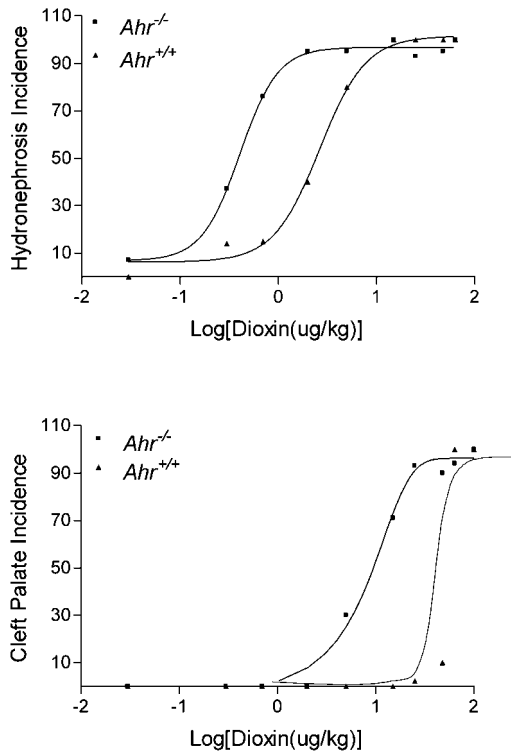


FIG. 2. **In vivo dose response of dioxin-induced terata.** Pregnant $Ahr^{+/+}$ dams mated to $Ahr^{-/-}$ males or $Ahr^{-/-}$ dams mated to $Ahr^{+/+}$ males were injected intraperitoneal with 0.03, 0.3, 0.7, 2.0, 5.0, 15.0, 25.0, 48.0, or 64.0 $\mu\text{g}/\text{kg}$ of dioxin at E10. At E18 the embryos were harvested and scored for hydronephrosis (*top panel*) and cleft palate (*bottom panel*). Each data point represents pooled data from at least three litters derived from three separate dams. Triangles represent progeny derived from $Ahr^{+/+}$ dams, and squares represent progeny derived from $Ahr^{-/-}$ dams. Curves were fit to a Sigmoidal dose response using nonlinear regression.

progeny from $Ahr^{-/-}$ dams to teratogenesis was the result of increased dioxin sensitivity of embryonic tissues, we took advantage of *in vitro* palate organ culture. The $Ahr^{+/+}$ embryos were harvested from their respective dams, and palatal shelves were placed into organ culture and dosed with various concentrations of dioxin. After 72 h, the palatal cultures were assessed for fusion and scored accordingly. We observed that palatal shelves harvested from either an $Ahr^{+/+}$ or an $Ahr^{-/-}$ responded identically at 0.3 nM, 1.0 nM, 3.3 nM, and 10 nM doses when cultured (Fig. 3).

Receptor Binding—To demonstrate that the receptor concentration in each of the $Ahr^{+/+}$ embryo cohorts was similar, we compared their ability to bind ligand. Photo affinity ligand binding experiments were performed on maternal and embryonic liver cytosols to examine the binding capacity of the receptor in $Ahr^{+/+}$ embryos from $Ahr^{+/+}$ or $Ahr^{-/-}$ dams. After normalization for protein concentration, we found no difference in the concentration of AHR in any of the experimental groups (Fig. 4).

Embryo Survival after Dexamethasone Treatment—To determine whether embryos from $Ahr^{-/-}$ dams were also more sensitive to compounds structurally unrelated to dioxins, we examined their response to dexamethasone. Dexamethasone is a known teratogen and fetotoxicant (27–34). We observed that at a maternal dose of 15 mg/kg of dexamethasone, embryos from $Ahr^{-/-}$ dams exhibited a 9-fold higher level of mortality than those from $Ahr^{+/+}$ dams ($p < 0.0006$, Fig. 5).

Dioxin Disposition in Embryonic Tissues—To test the idea that the embryos derived from $Ahr^{-/-}$ dams were receiving a higher dose of dioxin, we dosed E14 pregnant females with 25

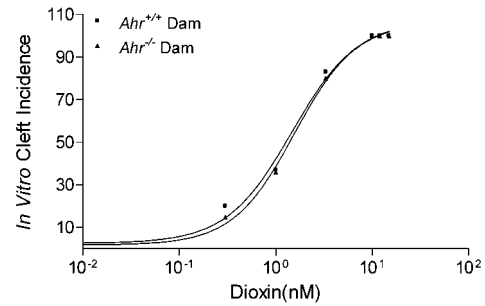


FIG. 3. **In vitro dose-response palatal shelf fusion.** Palates from E13 embryos were cultured for 72 h in the presence of Me_2SO , 0.3, 1.0, 3.3, or 10 nM of dioxin in Me_2SO . Palate shelves fixed in 10% formalin and counterstained with hematoxylin for visualization. Palate organ cultures were scored for fusion. Triangles represent $Ahr^{+/+}$ progeny derived from $Ahr^{-/-}$ dams, and squares represent $Ahr^{+/+}$ progeny derived from $Ahr^{+/+}$ dams.

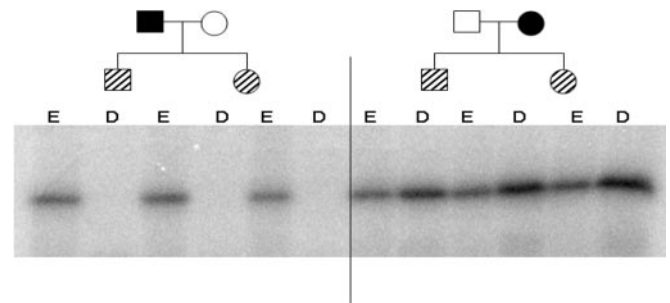


FIG. 4. **Photo affinity labeling to examine receptor binding affinity.** The livers were dissected from E18 embryos, and the cytosolic fraction from each litter was pooled and subjected to photo affinity labeling. The *left side* of the autoradiograph represents receptor isolated from $Ahr^{+/+}$ embryo pooled cytosols (E) and the $Ahr^{-/-}$ dam cytosol (D). Note that $Ahr^{-/-}$ dams lack functional AHR, and thus little ligand binding occurs, $0.4 \times 10^3 \pm 0.04$ DPM. The *right side* of the autoradiograph represents receptor isolated from pooled $Ahr^{+/+}$ embryo cytosols (E) and the $Ahr^{+/+}$ dam (D) cytosol. The $Ahr^{+/+}$ dams have functional AHR binding of $5.3 \times 10^3 \pm 1.0$ DPM. There was no significant difference in receptor binding between embryo samples from an $Ahr^{-/-}$ dam, $2.7 \times 10^3 \pm 0.3$ DPM versus embryo samples from an $Ahr^{+/+}$ dam, $2.8 \times 10^3 \pm 0.3$ DPM. All of the values are normalized for protein concentration.

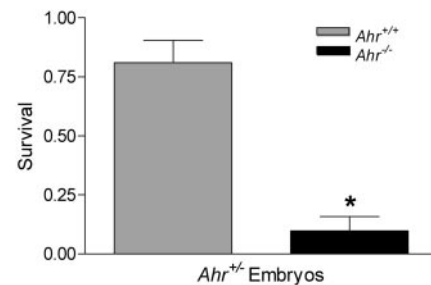


FIG. 5. **Pregnant females were dosed at E12 with 15 mg/kg dexamethasone in Me_2SO .** Three $Ahr^{+/+}$ dams gave rise to 14 live and 4 dead embryos, whereas $Ahr^{-/-}$ dams gave rise to 3 live and 25 dead embryos ($p < 0.0006$). Statistical analysis was performed using the *t* test (54).

$\mu\text{g}/\text{kg}$ of dioxin (intraperitoneal). At E15 the $Ahr^{+/+}$ embryos were harvested, and the amount of dioxin was assessed by mass spectrometry (26). We found that embryos derived from $Ahr^{-/-}$ dams had dioxin body burdens ~ 3 -fold greater than embryos derived from $Ahr^{+/+}$ dams, $p < 0.05$ (Fig. 6, *top panel*). An independent analysis of dioxin disposition was performed using a radiometric approach. In this experiment, we intraperitoneally dosed E15 pregnant females with 15 μCi of [^3H]TCDD. This dose is $\sim 25 \mu\text{g}/\text{kg}$ of dioxin, the same dose used in the mass spectrometry analysis. At E16 the $Ahr^{+/+}$

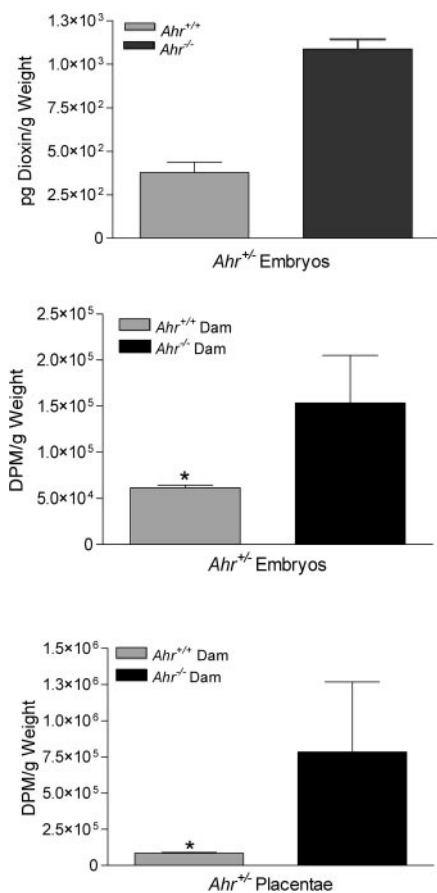


FIG. 6. Analysis of dioxin body burden in embryo cohorts. *Top panel*, mass spectrometry analysis. Pregnant dams were dosed with 25.0 $\mu\text{g}/\text{kg}$ of dioxin at E14. The embryos were harvested at E15, and dioxin was quantified by mass spectrometry. The experimental cohort consisted of three pooled litters from each of the genotypic crosses performed in triplicate. The gray column represents *Ahr*^{+/+} embryos from *Ahr*^{+/+} dams. The black column represents *Ahr*^{-/-} embryos from *Ahr*^{-/-} dams ($p < 0.03$). *Middle and bottom panels*, [³H]TCDD analysis. Pregnant dams were dosed with 15 μCi of [³H]TCDD at E15. The embryos and placentae were harvested at E16, and [³H]TCDD was measured by scintillation counting. *Middle panel*, the *Ahr*^{-/-} embryos from *Ahr*^{-/-} dams have a 3-fold greater body burden of dioxin ($p < 0.0226$). *Bottom panel*, the *Ahr*^{-/-} placentae from *Ahr*^{-/-} dams have a 9-fold greater burden of dioxin ($p < 0.05$). All of the statistical analyses were performed using the *t* test (64).

embryos and placentae were harvested and dissolved in tissue solubilizer, and radioactivity was measured. After normalization to tissue weight, we found that embryos derived from *Ahr*^{-/-} dams had dioxin body burdens that were ~3-fold greater than in embryos derived from *Ahr*^{+/+} dams ($p < 0.05$; Fig. 6, *middle panel*). In the radiometric study, we also examined the [³H]TCDD levels in individual placentae of the embryos from each progeny cohort. After normalization to tissue weight, we found a 9-fold greater concentration of [³H]TCDD in the placentae of embryos derived from an *Ahr*^{-/-} dam ($p < 0.05$; Fig. 6, *bottom panel*).

Dioxin Disposition in Maternal Tissue—To determine whether maternal disposition of dioxin was influenced by genotype at the *Ahr* locus, we employed the radiometric method and assessed levels of dioxin in various major organs of exposed dams. We found that the livers of *Ahr*^{+/+} dams retained 60% of the total dioxin dose compared with 8% of the total dose in the livers of *Ahr*^{-/-} dams ($p < 0.0001$; Fig. 7). There was significantly more dioxin in the skin, thymus, and lung of *Ahr*^{-/-} versus *Ahr*^{+/+} dams. In all other tissues there were no significant differences in dioxin levels.

Dexamethasone Disposition in Embryonic Tissues—To test the idea that the embryos derived from *Ahr*^{-/-} dams were receiving a higher dose of dexamethasone, we dosed E16 pregnant females with 15 μCi of [³H]dexamethasone. Two hours later, *Ahr*^{+/+} embryos, and placentae were harvested and dissolved in tissue solubilizer, and radioactivity was measured. After normalization to tissue weight, we found that embryos derived from *Ahr*^{-/-} dams had dexamethasone body burdens that were 2–3-fold greater than in embryos derived from *Ahr*^{+/+} dams ($p < 0.0001$; Fig. 8, *top panel*). In the radiometric study, we also examined the [³H]dexamethasone levels in individual placentae of the embryos from each progeny cohort. After normalization to tissue weight, we found nearly a 3-fold greater concentration of [³H]dexamethasone in the placentae of embryos derived from an *Ahr*^{-/-} dam ($p < 0.0001$; Fig. 8, *bottom panel*).

DISCUSSION

The AHR is a ligand-activated transcription factor that plays multiple roles in mammalian biology. In response to certain polycyclic aromatic hydrocarbons, the AHR up-regulates a battery of xenobiotic metabolizing enzymes, such as cytochromes P450, 1A1, 1A2, and 1B1 (14, 15, 36–38). The induced P450s play two roles in xenobiotic metabolism. First they oxidize a variety of polycyclic aromatic hydrocarbons to more polar and excretable forms (39, 40). Second, they can serve as inducible sinks for recalcitrant substrates such as dioxin, thus markedly influencing their disposition (41, 42). Because this system commonly results in a substrate inducing its own metabolism, we refer to it as an adaptive response pathway (43).

When more potent agonists, like dioxin, bind to the receptor, the adaptive pathway is up-regulated, as well as an additional battery of toxic responses (9, 38, 45). Hydronephrosis and cleft palate are classic end points of dioxin-induced teratogenesis (8, 9, 47, 48). The observation that the AHR is highly expressed in the urogenital tract and craniofacial tissues of the developing embryo supports the idea that dioxin-induced terata are largely a tissue autonomous process (49). Additional evidence to support this idea comes from palate culture experiments where fusion between two palatal shelves can be blocked by exposure to dioxin *in vitro* (50). Recent evidence also supports the concept that the toxicity/teratogenesis associated with dioxin exposure is mediated through the action of AHR within the nucleus, presumably transcriptional (51).

The AHR has also been shown to play an important role in normal development (16, 18, 19). Mice that harbor a null allele at the *Ahr* locus display reduced liver weights and subtle vascular changes in a number of organs (16, 19, 52, 53). The most prominent vascular abnormality reported in these nulls is the presence of a patent *ductus venosus* (DV) throughout life (53). The DV is a porto-systemic shunt that allows blood to bypass the hepatic circulation during embryogenesis (55–57). In the *Ahr*^{-/-} mouse, the DV allows a significant fraction of the portal blood supply to bypass liver perfusion and exit through the inferior vena cava. The observation that *Ahr*^{-/-} mice exhibit a patent DV coupled to their demonstrated inability to mount a metabolic defense against certain xenobiotics provided early suggestions that these mice would display altered xenobiotic disposition, especially for those xenobiotics cleared by the liver and metabolized by cytochromes P4501A monooxygenases.

Evidence for the Role of Maternal AHR in Dioxin-induced Terata—The initial focus of this investigation was to determine whether there was any contribution of the maternal AHR on the teratogenic response of the embryo to dioxin. To this end, we utilized the *Ahr*^{-/-} mouse model. In one set of two crosses, we mated the *Ahr*^{-/-} female to an *Ahr*^{+/+} male. In the second cross, an *Ahr*^{+/+} female was mated to an *Ahr*^{-/-} male. We then

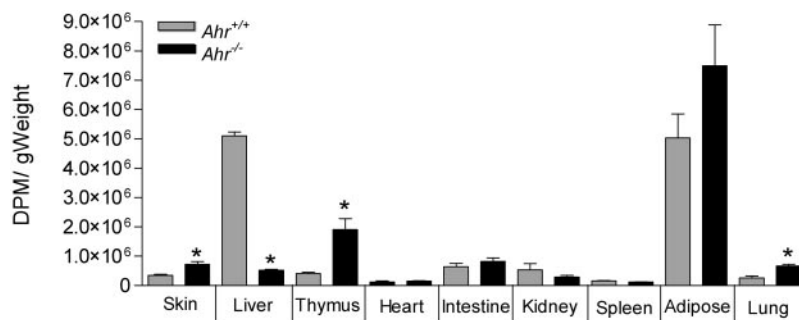


FIG. 7. **Analysis of adult female dioxin burden in several tissues.** Dams were dosed with 15 μCi of [^3H]TCDD. After 24 h, the dams were sacrificed, and the tissues were removed. Tissues from 3 *Ahr*^{+/+} and 3 *Ahr*^{-/-} dams were assessed for radioactivity. After normalization to tissue weight, the *Ahr*^{+/+} dams had significantly more dioxin contained in their liver than the *Ahr*^{-/-} dams ($p < 0.0001$). More dioxin localized to the skin ($p < 0.02$), thymus ($p < 0.008$), and lung ($p < 0.08$) of the *Ahr*^{-/-} dam when compared with the *Ahr*^{+/+} dam. All of the statistical analyses were performed using the *t* test (64).

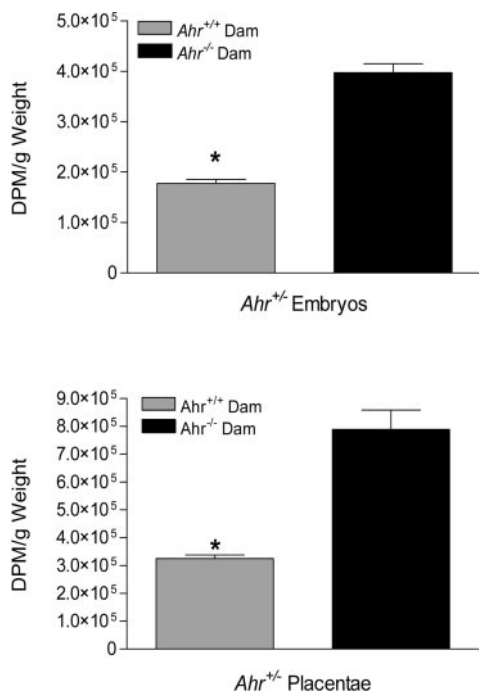


FIG. 8. **Analysis of dexamethasone body burden in embryo cohorts.** The dams were dosed with 15 μCi of [^3H]dexamethasone intraperitoneally. After 2 h, the dams were sacrificed, and the tissues were removed. Embryo and placental tissues from three *Ahr*^{+/+} and two *Ahr*^{-/-} dams were assessed for radioactivity. After normalization to tissue weight, the embryos and placentae from an *Ahr*^{-/-} dam had significantly more dexamethasone than embryos from *Ahr*^{+/+} dams ($p < 0.0001$). All of the statistical analyses were performed using the *t* test (64).

compared induction of terata in the two progeny cohorts that were genetically identical at the *Ahr* locus (*i.e.* *Ahr*^{+/+}). Through our analysis, we observed that offspring from *Ahr*^{-/-} dams were 5-fold more sensitive to dioxin-induced cleft palate and hydronephrosis (Fig. 2). Based upon these results, it was clear that a maternal *Ahr* null allele had a significant influence on dioxin teratogenicity.

Altered Disposition of Dioxin—To explain the increased sensitivity, we hypothesized that more dioxin was reaching the embryos in *Ahr*^{-/-} dams. This idea was supported by the observation of a “left shift” in the dose-response curves for both the cleft palate and hydronephrosis end points (Fig. 2). Importantly, this equivalent left shift occurred for two independent processes, responding at markedly different dioxin doses. That is hydronephrosis is 100% penetrant at 15 $\mu\text{g}/\text{kg}$ dioxin, whereas cleft palate formation is not fully penetrant until 64

$\mu\text{g}/\text{kg}$ dioxin. To examine disposition, we quantified the amount of dioxin that reached the embryo from each of the maternal genotypes. To this end, we employed both mass spectrometry and radiometric measurements. Using both of these methods, we found that there was ~3-fold more dioxin reaching the embryos from an *Ahr*^{-/-} dam (Fig. 6, *top* and *middle* panel). A 9-fold greater concentration of dioxin was measured in the placentae of *Ahr*^{+/+} embryos from *Ahr*^{-/-} versus *Ahr*^{+/+} dams (Fig. 6, *bottom* panel).

It has been well established that dioxin crosses the placental barrier and reaches the embryo (58, 59). Previous experiments have measured the embryo dioxin levels between 0.04 and 0.14% of the maternal dose/gram of tissue (59). In these earlier experiments, the dam retains between 30 and 40% of the dose/gram of tissue, with the liver retaining the highest percentage of dioxin, 10% (59). The data presented here largely agree with these earlier reports, with dioxin levels in *Ahr*^{+/+} embryos being found to lie between 0.15 and 0.26% of the maternal *Ahr*^{+/+} dose/gram of tissue. Importantly, disposition to the embryos from the maternal null was higher, lying in the range of $0.41 \pm 0.12\%$ of the maternal dose/gram of tissue. Similarly, the *Ahr*^{+/+} dam retained $64 \pm 4\%$ of the dose/gram in the liver, whereas the *Ahr*^{-/-} dam retained only $8 \pm 1\%$ of the dose/gram in the liver. The *Ahr*^{-/-} dams retained the highest dose of dioxin in the adipose tissue at ~23% of the dose/gram of adipose tissue, which was not statistically different from the amount measured in the *Ahr*^{+/+} dams.

Increased Sensitivity to Dexamethasone—The dioxin experiments were initially designed to examine the role of the maternal AHR in the formation of dioxin-induced terata. In an attempt to delve more deeply into the mechanism for this sensitivity, we administered the glucocorticoid, dexamethasone. Dexamethasone is a known fetotoxicant that does not bind the AHR (60). Our approach was to compare the fetotoxic response to dexamethasone in progeny from *Ahr*^{+/+} versus *Ahr*^{-/-} dams. This experiment was performed to determine whether the relationship between the *Ahr* locus and increased chemical sensitivity extended beyond AHR agonists. What we found was that the embryos from *Ahr*^{-/-} dams were 9-fold more sensitive to dexamethasone treatment. This experiment suggests that *Ahr*^{-/-} dams provide less efficient protection of their developing offspring from a spectrum of chemical teratogens, even those that are structurally unrelated to AHR agonists.

Altered Disposition of Dexamethasone—One of our hypotheses is that the DV results in an ineffective maternal barrier to chemical teratogens. To test this idea, the disposition of dexamethasone, a fetotoxicant that does not bind directly to the AHR, was examined. To this end, we quantified the amount of dexamethasone that reached the embryo from each of the maternal genotypes. We employed radiometric methods and found

that there was 2–3-fold more dexamethasone reaching the embryos from an *Ahr*^{-/-} dam (Fig. 8, top panel). A 2.7-fold greater concentration of dexamethasone was measured in the placenta of *Ahr*^{+/-} embryos from *Ahr*^{-/-} versus *Ahr*^{+/+} dams (Fig. 8, bottom panel).

Our disposition studies strongly support the idea that the higher embryonic sensitivity to both dioxin and dexamethasone is the result of an ineffective maternal barrier. This inefficiency could result from decreased hepatic or first pass clearance of chemical teratogens in the maternal compartment. Two observations regarding *Ahr*^{-/-} mice lead us to this hypothesis. The first is a substantial porto-caval shunt, known as the DV, present in essentially all *Ahr*^{-/-} mice. Such shunting would be predicted to have a profound effect on a broad spectrum of chemical teratogens. Second, *Ahr*^{-/-} mice display a marked reduction in inducible CYP1A monooxygenases. Given that these P450s can act as sinks or degrading enzymes for certain chemical teratogens, their decreased expression could also play a significant role in teratogen disposition and availability to the embryo compartment. The bottom line is that one or both of these mechanisms could result in a higher concentration of dioxin, dexamethasone, or any number of teratogens.

Initially, we predicted that if altered disposition was at the heart of increase embryo sensitivity, then removal of the maternal variable should result in embryos exhibiting similar sensitivity to dioxin. To test this idea, we performed palate culture experiments that allowed the dosing of the developing organ *in vitro*, eliminating the influence of the maternal compartment. By scoring “failure to fuse” as an end point, we observed identical dioxin dose-response curves for palates obtained from embryos derived from either an *Ahr*^{+/+} or an *Ahr*^{-/-} dam. This demonstrates that the maternal component is critical in determining the susceptibility of her offspring to dioxin-induced terata. As a supporting experiment, we characterized the AHR found in the embryonic palate derived from *Ahr*^{+/+} and *Ahr*^{-/-} dams to determine whether they bound ligand with the same capacity. We found ligand binding to be indistinguishable, suggesting that receptor biochemistry in the embryo was not directly affected by maternal genotype.

Conclusions—Two important conclusions can be drawn from our results. First, these experiments provide one of the most compelling proofs that the maternal genotype harbors considerable influence over the teratogenic action of chemical agents. Although polymorphisms in genes important in the maternal barrier have been proposed, strong examples in the literature are rare (35, 44, 46). Second, the *Ahr*^{-/-} mutant may provide a powerful system for the identification of chemical teratogens. Given that hepatic first pass and maternal clearance are both significant modifiers of pharmacological action, this model may extend to numerous biologically active compounds where experimental studies are enhanced when the agent under study is not sequestered or metabolized.

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