Region-Specific Inhibition of Prostatic Epithelial Bud Formation in the Urogenital Sinus of C57BL/6 Mice Exposed in Utero to 2,3,7,8-Tetrachlorodibenzo-p-dioxin

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In utero 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) exposure causes abnormal ventral, dorsolateral, and anterior prostate development in wild-type but not aryl hydrocarbon receptor (AhR) null mutant C57BL/6 mice. Experiments have now been conducted to test the hypothesis that TCDD causes an AhR-dependent inhibition of the earliest visible stage of prostate development, the formation of prostatic buds by urogenital sinus (UGS) epithelium. A novel method for viewing budding was developed that uses scanning electron microscopy of isolated UGS instead of three-dimensional reconstruction of serial histological sections of intact UGS. In the initial experiment, the time course for prostatic epithelial bud formation in vehicle- and TCDD-exposed wild-type C57BL/6J mice was determined. A single maternal dose of TCDD (5 μg/kg) on gestation day 13 delayed the appearance of dorsal, lateral, and anterior buds by about one day, reduced dorsolateral bud number, and prevented ventral buds from forming. No such effects were seen in TCDD-exposed AhR null mutant fetuses, while AhR null mutation, alone, had no detectable effect on budding. Treatment of wild-type dams with sufficient 5α-dihydrotestosterone (DHT) to masculinize female fetuses failed to protect against the inhibition of budding caused by TCDD. These results demonstrate that in utero TCDD exposure causes an AhR-dependent inhibition of prostatic epithelial bud formation commensurate with its inhibitory effects on ventral and dorsolateral prostate development, and that the inhibition of budding is not due to insufficient DHT. Inhibited bud formation appears to be the primary cause of abnormal prostate development in TCDD-exposed mice.

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In utero and lactational 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) exposure causes abnormal prostate development in rats at doses close to background body burdens of TCDD- equivalents in humans (reviewed by Theobald et al., 2003). In mice, the developing prostate is also vulnerable to TCDD (Lin et al., 2002a; Sommer and Peterson, 1997; Theobald and Peterson, 1997). A single maternal dose of 5 μg TCDD/kg on gestation day (GD) 13 inhibited ventral, dorsolateral, and anterior prostate growth in C57BL/6 mice (Lin et al., 2002a). Reductions in ventral prostate weight were far more severe than reductions in dorsolateral prostate weight, while anterior prostate weight was intermediate in sensitivity. Expression of mRNA for major secretory proteins characteristic of each lobe was relatively unaffected (relative to cyclophilin mRNA expression) in the dorsolateral prostate but was inhibited in the anterior prostate and severely inhibited in the ventral prostate. The effects described above were seen in wild-type (Ahr+/−) mice but not aryl hydrocarbon receptor (AhR) null mutant (Ahr−/−; AhRKO) mice. Consequently, effects of TCDD on prostate development are dependent on the presence of AhR, a ligand-activated transcription factor that mediates most effects of TCDD and related ligands, and which appears to have diverse functions and to interact with multiple other signaling pathways (Carlson and Perdew, 2002; Gasiewicz and Park, 2003).

In utero and lactational TCDD exposure also inhibits ductal branching morphogenesis. In the dorsal and lateral prostate lobes of TCDD-exposed C57BL/6J mice, the number of main ducts was reduced but ductal branching appeared to be unaffected. In the anterior prostate, main duct number was unchanged but branching was severely inhibited. And in the ventral prostate, no ductal structure was present (Ko et al., 2002). The critical windows of vulnerability to TCDD have also
been studied (Lin et al., 2002b). Effects of TCDD on ventral prostate development in C57BL/6J mice began before GD 16 and were due primarily to exposure between GD 13 and birth. Dorsolateral prostate development was inhibited about equally by in utero and by lactational TCDD exposure, and vulnerability did not begin until GD 16. Anterior prostate development was also affected by both in utero and lactational TCDD exposure, particularly the former: vulnerability began before GD 16 and continued into postnatal life. These results demonstrate that TCDD inhibits the very earliest stages of prostate development, and strongly suggest that TCDD does so, at least in part, by acting on the tissue from which the prostate develops: the urogenital sinus (UGS).

The initial visible step in prostate formation is the outgrowth of buds from UGS epithelium into the surrounding mesenchyme. Prostatic epithelial bud formation and growth are androgen dependent. In mice, budding begins on GD 16 or GD 17 (when GD 0 is the day after overnight mating) and is complete before birth. Some buds regress but most elongate, become canalized, and ultimately develop into the various prostate lobes: dorsal and lateral buds into dorsolateral prostate, ventral buds into ventral prostate, and anterior buds into anterior prostate (Brewer, 1962; Cunha et al., 1987; Lasnitzki and Mizuno, 1980; Lung and Cunha, 1981; Raynaud, 1942, 1962). The hormonal, cellular, and molecular control of UGS and prostate development has recently been reviewed (Marker et al., 2003).

Bud formation by the UGS has traditionally been studied by analysis of stained histological sections. Three-dimensional computer-assisted serial section reconstruction was first used to study UGS development a decade ago (Timms et al., 1994). This technique has contributed to our understanding of both normal prostate development in rodents and humans (Timms et al., 1994) and how in utero TCDD exposure impairs prostate development in the rat (Roman et al., 1998; Timms et al., 2002), but it is time-consuming and labor-intensive. One objective of our research, therefore, was to determine if scanning electron microscopy (SEM) of isolated UGS epithelium could be used as a higher throughput method to study the ontogeny of prostatic epithelial bud formation. When this proved feasible, we conducted experiments to test the hypothesis that in utero TCDD exposure causes an AhR-dependent inhibition of prostatic epithelial bud formation in the mouse commensurate with its differential effects on subsequent development of the various prostate lobes. And when TCDD was found to inhibit the androgen-dependent budding process, we determined if 5α-dihydrotestosterone (DHT) can protect against this inhibition.

MATERIALS AND METHODS

Animals. Most mice were from a C57BL/6J breeding colony, founded and maintained with mice purchased from The Jackson Laboratory (Bar Harbor, ME). Others were from an AhRKO breeding colony established with mice (Schmidt et al., 1996) generously provided by Dr. Christopher A. Bradfield (Department of Oncology, University of Wisconsin). The AhRKO colony was backcrossed to pure C57BL/6J mice for 10 to 12 generations before these experiments were conducted. Possible contributions of different genetic backgrounds to differences between treatments were controlled by dosing comparable percentages of dams with TCDD and vehicle at each stage of backcrossing.

Mice were housed in clear plastic cages with heat-treated, chippered-aspen bedding in animal rooms kept at 24°C and 50 ± 4% relative humidity and lighted from 0600 to 1800 h. Feed (5015 Mouse Diet, PMI Nutrition International, Brentwood, MO) and tap water were available ad libitum. All procedures were approved by the University of Wisconsin Animal Care and Use Committee.

Mice were bred by housing females (90 to 120 days of age) overnight with males. For experiments that required AhRKO fetuses, AhR heterozygous (Ahr+/−) females were mated with Ahr+/− males. The day after mating was considered to be GD 0.

Treatments. Pregnant mice were given a single oral dose of TCDD (5 µg/kg) or vehicle (95% corn oil/5% acetone, 5 ml/kg) on GD 13. In one experiment, pregnant mice were implanted on GD 12 with either a sustained-release pellet designed to release 15 mg DHT over 90 days (Innovative Research of America, Sarasota, FL) or with the corresponding placebo pellet. Pellets were implanted subcutaneously under isofluorine anesthesia.

Necropsies and analytical procedures. Dams were euthanized by CO2 overdose. Fetuses were removed, placed in ice-cold phosphate-buffered saline, and bisected. Sex was determined by gonadal inspection. When necessary, a tail sample of blood was taken for genotyping by polymerase chain reaction analysis, as previously described (Benedict et al., 2000).

UGS complexes were removed from male fetuses and subjected to limited trypsin digestion to separate epithelium from mesenchyme, using procedures similar to those described by Cunha and Donjacour (1987). Briefly, UGSs were incubated in calcium- and magnesium-free Hanks’ balanced salt solution (HBSS) containing 1% trypsin at 4°C (GibcoBRL, Grand Island, NY). After 90 min, UGSs were washed twice with HBSS and incubated for 5 min in HBSS plus 5% charcoal/dextran-stripped fetal bovine serum to attenuate any remaining trypsin activity. UGS mesenchyme was then separated from UGS epithelium, using forceps under a dissecting microscope. As shown in Figure 1A, the UGS appears as an opaque nondescript object bordered by the bladder, urethra, and seminal vesicles. Removal of mesenchyme reveals the underlying epithelium, with some of the larger buds visible, in profile, by light microscopy (Fig. 1B; note higher magnification). The solid line in Figure 1A is an outline of the UGS epithelium drawn at the same magnification as the photograph of intact UGS.

Samples of UGS epithelium were fixed overnight in calcium- and magnesium-free HBSS containing 2.5% glutaraldehyde, dehydrated through a graded series of ethanol to 100%, and dried by the critical-point procedure using liquid CO2 as the transitional fluid. Dried UGS specimens were mounted on aluminum stubs with double stick conductive carbon tape, coated with a thin layer of gold using a sputter-coater (Auto Conductavac IV, See Vac Inc., Pittsburgh PA), and examined at an accelerating voltage of 5 kV on a Hitachi S-570 scanning electron microscope (Hitachi, Tokyo, Japan). Images were captured with a digital capture system (Gatan, Pleasanton, CA), and examined at an accelerating voltage of 5 kV on a Hitachi S-570 scanning electron microscope (Hitachi, Tokyo, Japan). Images were captured with a digital capture system (Gatan, Pleasanton, CA). Prostatic epithelial bud numbers were determined using images taken at multiple angles. Bud counts were not affected by the presence of broken buds because the proximal portions of these buds remained. Prostatic buds were usually well separated from urethral buds, but if they were contiguous, buds were not counted if they were located on the urethra (i.e., where opposite epithelial surfaces are parallel to each other). There were at least three samples per time, treatment, and genotype.

Serum DHT concentrations were determined by radioimmunoassay, using antibody from Endocrine Sciences (Calabasas Hills, CA) and [1,2,4,5,6,7–3H] DHT from Amersham Biosciences (Piscataway, NJ), as previously described (Loeffler and Peterson, 1999).

Statistical analysis. Analyses were conducted with the litter as the experimental unit. UGS samples were processed from all male fetuses, and if data
were obtained from more than one male per genotype from any given litter, results were averaged prior to statistical analysis. Parametric analyses were conducted on untransformed, square root transformed, and ranked data. For data that passed Levene’s test for homogeneity of variance and which appeared to be normally distributed, analysis of variance (ANOVA) was conducted. If a significant effect was found, the least significant difference test was used to determine which group(s) differed from the appropriate control group. Data were also analyzed by the Kruskal-Wallis nonparametric ANOVA, with post hoc testing by the distribution-free multiple-comparison test. Significance was set at $p < 0.05$. Results are presented as means ± SE.

RESULTS

Overview of UGS Epithelial Structure

The three-dimensional structure of UGS epithelium, and the position and shape of the prostatic buds that develop from it, can be visualized by SEM. Four views of representative UGS epithelium from a vehicle-exposed GD 15 wild-type male fetus, taken at successive 90° rotations of the sample, are shown in Figure 2. All major structural elements can be readily discerned at this time because prostatic buds have not yet formed. UGS epithelium is attached to the Wolffian ducts, bladder (at the bladder neck), and urethra, as shown. Müllerian ducts are typically lost during sample preparation, but the sites at which they had been attached can be seen (arrowheads). The overall shape of UGS epithelium on GD 15 remains similar from this time through birth, except that it becomes proportionately longer between the Wolffian ducts and bladder.

Müllerian ducts were occasionally present in imaged UGS epithelial samples obtained on GD 15, 16, or 17. One such example, from a vehicle-exposed GD 15 wild-type male, is shown in Figure 3. The prostatic utricle, a vestigial tissue formed from the regressing Müllerian duct, could be seen in some UGS samples on GD 18 and 19 (not shown). Another developmental change is in the Wolffian ducts, which differentiate by GD 17 into ejaculatory ducts (between the UGS and seminal vesicles) and ductus deferens (between seminal vesicles and epididymides).

Quantitative analysis of budding patterns required at least four images of each fixed UGS epithelium. Lateral views often revealed asymmetry in budding patterns. Figure 4 shows two lateral views (A and B), one view (C) of ventral buds, and one view (D) of dorsal and anterior buds of representative UGS epithelium from a vehicle-exposed wild-type C57BL/6J mouse on GD 18. Most buds remained intact, although the distal portion of others broke off during sample processing, and most could be identified with confidence (based on their position) as being ventral, dorsal, anterior, or lateral buds. On GD 18, ventral buds (tinted blue in Fig. 4) are shaped like elongated cylinders that often have a slightly enlarged distal end. Dorsal buds (green) are somewhat shorter than ventral buds and are cylindrical at the base but usually widen into a bulbous top. Anterior buds (red) are similar in shape to dorsal buds but are typically much larger. Lateral buds (yellow) also resemble dorsal buds but are typically longer. The remaining prostatic epithelial buds are located on the lateral surfaces of the UGS. These buds (tinted blue-green) are generally short on GD 18 and are difficult to classify as either dorsal or lateral, because there is no distinct boundary between them and the adjoining buds that are clearly dorsal or clearly lateral.

Ontogeny of Prostatic Epithelial Buds in Vehicle and TCDD-Exposed Wild-Type Mice

UGS development and prostatic epithelial bud formation were examined at 24-h intervals from GD 14 through birth. Fetuses were from pregnant wild-type C57BL/6J mice dosed on GD 13 with 5 μg TCDD/kg or vehicle.

In vehicle-exposed fetuses, prostatic buds were not yet present by GD 14, but UGS epithelium had already developed most of its characteristic shape (Fig. 5A). The bladder neck and the shallow mounds on the dorsal surface of the UGS from which anterior buds subsequently develop first become evident in most samples on GD 15. All remaining features of UGS epithelial structure, other than buds, were present by this time (Fig. 5B). In most samples, little difference was seen in the structure of UGS epithelium between GD 15 and GD 16 (Fig.
5C); however, dorsal and/or anterior buds were seen in some samples on GD 16. When present, these buds were few in number and generally short. On GD 17, dorsal and anterior buds were present in every UGS examined, ventral buds were present in most samples, and lateral buds were present in some (Fig. 5D). By GD 18, the numbers of dorsolateral, anterior, and ventral buds had reached their maximum, and bladder neck extension was complete (Fig. 5E). Most buds continued to lengthen through the end of the study on GD 19/postnatal day (PND) 0 (Fig. 5F).

In utero TCDD exposure (5 μg/kg on GD 13) had no detectable effect on UGS structure in wild-type mice on GD 14 (Fig. 5G), but it delayed the start of bladder neck extension and the appearance of the shallow mounds from which anterior buds subsequently develop from GD 15 (Fig. 5H) to GD 16 (Fig. 5I). In contrast to vehicle-exposed UGS epithelium, ventral, lateral, and anterior buds were entirely absent from UGSs from TCDD-exposed mice on GD 17 (Fig. 5J), and only about half had any dorsal buds. When dorsal buds were present on GD 17 they were generally few in number and shorter than they were wide. GD 18 was the first day on which prostatic epithelial buds were prominent in TCDD-exposed UGS (Fig. 5K). Dorsal and anterior buds were present in all samples examined, and buds were present on the lateral surfaces of the UGS in some, but ventral buds were absent from all samples. Ventral buds were still absent from the UGS of TCDD-exposed wild-type mice on GD 19/PND 0, and lateral surfaces had few if any buds (Fig. 5L).

**Effects of AhR Null Mutation on UGS Epithelial Structure and Bud Number in Vehicle- and TCDD-Exposed Fetuses**

The UGS from each vehicle-exposed wild-type mouse had eight ventral buds on GD 18 (Figs. 6A, 7), and an average of 52 “dorsolateral” buds (Fig. 7). Although many buds could be readily identified as either dorsal or lateral, neither dorsal nor lateral buds could be counted reliably due to the fact that each group is in immediate proximity to another group of similarly shaped buds on the lateral surface of the UGS. Dorsolateral-bud numbers are the total number of dorsal buds (tinted green in Fig. 4) plus lateral buds (yellow) plus buds of uncertain nature on the lateral surfaces of the UGS (blue-green).

AhR null mutation, alone, had no detectable effect on the appearance of buds in any region of the UGS (Fig. 6B) or on the number of ventral or dorsolateral buds (Fig. 7) on GD 18. In contrast, in utero exposure to a single maternal dose of TCDD (5 μg/kg on GD 13) completely prevented ventral bud formation in wild-type mice, and reduced the number of dorsolateral buds by about 25% (Fig. 7). The physical appearance of dorsal buds did not appear to be affected, but few buds were present on the lateral surfaces of the UGS. When present, buds on the lateral surfaces were generally smaller in TCDD-exposed wild-type mice (Fig. 6C) than in vehicle-exposed wild-type mice. TCDD had no detectable effect in AhRKO mice on the physical appearance of buds in any region of the UGS (Fig. 6D) or on the number of ventral or dorsolateral buds (Fig. 7).

Neither TCDD exposure nor AhR null mutation, alone or in...
combination, had any detectable effect on the number of anterior buds (typically five or six per UGS) or on their morphology, except that anterior buds appeared to be smaller in TCDD-exposed wild-type mice than in the other three groups.

Effects of in Utero DHT Exposure on UGS Epithelial Structure in Vehicle- and TCDD-Exposed Wild-Type Fetuses

To determine whether the impairment in prostatic epithelial budding caused by in utero TCDD exposure can be prevented by DHT, sustained-release DHT-containing or placebo pellets were implanted sc in pregnant mice on GD 12. Dams were given TCDD (5 μg/kg) or vehicle on GD 13, and budding was examined on GD 18. DHT treatment was sufficient to elevate maternal DHT concentrations to about 5 ng/ml serum on GD 18 (not shown) and to strongly masculinize female fetuses, as shown by male-like external genitalia (not shown) and substantially increased anogenital distance (not shown).

UGS epithelial structure and prostatic budding in male fetuses exposed to vehicle and placebo pellets were essentially the same (Fig. 8A) as in vehicle-exposed fetuses from experiments without pellets. In utero TCDD exposure prevented ventral bud formation, caused buds to be largely absent from the lateral surfaces of the UGS, and reduced dorsolateral bud number to 66% of the control value in fetuses exposed to placebo pellets (Figs. 8B and 9). In utero DHT exposure had no significant effect on the number or appearance of ventral, dorsolateral, or anterior prostatic buds in either vehicle- or TCDD-exposed males or on the pattern of effects caused by TCDD (Figs. 8C and 8D, respectively; Fig. 9).

DISCUSSION

SEM as a Technique for Studying Prostatic Epithelial Bud Formation

SEM of isolated UGS epithelium proved to be an effective method for observing the development of prostatic buds. Not only is it a practical method of processing relatively large numbers of UGS samples, but it provides substantially better resolution of bud structure than is possible with three-dimensional reconstructions of serial histological sections, where resolution is limited by section thickness. The SEM approach has several limitations, however. It only reveals the outer structure of UGS epithelium, the distal part of some buds break off during sample processing, it is difficult to consistently mount samples in the same orientation, bud volumes cannot be quantitated, and it is still not a rapid technique. Nevertheless, SEM of isolated UGS epithelium appears to be the most efficient method for visualizing the ontogeny of prostatic epithelial buds and for characterizing the effects of exogenous chemicals on this process. Although many samples were lost in the initial stages of this research, nearly all resulted in usable SEMs once an investigator gained experience with sample mounting. This method allowed us to conduct a substantially more comprehensive set of observations than would have otherwise been possible with traditional methods. Development of this novel technique should facilitate studies into possible connections between abnormalities in prostatic ontogeny and prostatic disease later in life. Specifically, SEM of isolated UGS epithelium is a practical way to determine whether chemicals cause abnormalities in prostatic epithelial budding and, if they do, to determine the experimental conditions under which such effects occur. The more time-consuming but generally more definitive technique, histological analysis of serial sections, can then be used to focus on the most critical times and doses.

The physical appearance of prostatic epithelial buds in vehicle-exposed mice, as seen by SEM, was consistent with that previously described by other researchers who used routine histological sections (with or without three-dimensional reconstruction of serial sections) to visualize the structure of mouse UGS and the development of prostatic epithelial buds from it (Brewer, 1962; Mauch et al., 1985; Raynaud, 1942, 1962;
Results were relatively consistent from one sample to another, particularly among replicate samples from the same litter and genotype. The timing of bud appearance was also consistent with previous reports. In control mice, prostatic epithelial bud formation is reported to begin on GD 16 or GD 17 and to be complete before birth, whereas seminal vesicles (which develop from Wolffian ducts) are reported to first appear on GD 15 or 16 (Brewer, 1962; Cunha et al., 1987; Lasnitzki and Mizuno, 1980; Lung and Cunha, 1981; Raynaud, 1942, 1962). In vehicle-exposed mice, we observed dilation of...
the Wolffian ducts on GD 15, and seminal vesicles were present on GD 16. We also found that dorsal and anterior buds first began to appear on GD 16, that ventral and lateral buds first appeared on GD 17, and that budding was complete by birth.

Relationship between Prostatic Epithelial Bud Formation and Ductal Morphogenesis

The present manuscript appears to be the first to report prostatic epithelial bud numbers in either UGS as a whole or in all regions of the UGS. This information yields new insights into the relationship between prostatic epithelial bud formation and ductal morphogenesis, a process originally characterized by Lung and Cunha (1981) and by Sugimura et al. (1986).

We previously reported that ventral, anterior, lateral, and dorsal prostates in vehicle-exposed adult C57BL/6J mice average six, four, six, and forty-two main ducts, respectively (Ko et al., 2002). Now that bud counts are available, it appears that six of the eight ventral buds typically develop into the main ducts of the ventral prostate, and that four of the five or six anterior buds typically develop into the main ducts of the anterior prostate. Buds identified as lateral (yellow in Fig. 4) are sufficient in number and size to account for the six main lateral prostatic ducts seen in adulthood. Consequently, it appears that the remaining buds on the lateral surfaces of the UGS (those tinted blue-green in Fig. 4) are not likely to develop into main ducts of the lateral prostate. These buds tend to be the shortest of all prostatic buds on GD 18, although most are substantially longer on GD 19, which suggests that many will ultimately develop into main ducts. The fact that buds identified as dorsal (green in Fig. 4) are insufficient in number to account for the presence of an average of 42 main ducts per dorsal prostate of control adult mice further suggests that many short buds on the lateral surface of the UGS ultimately develop...

FIG. 5. Lateral views of UGS epithelium from vehicle- (A–F) and TCDD-exposed (G–L) wild-type mice on GDs 14, 15, 16, 17, 18, and 19/PND 0, respectively. Dams were dosed orally on GD 13 with 5 μg TCDD/kg or vehicle. Dorsal buds (DB), lateral buds (LB), ventral buds (VB), and anterior buds (AB) are as shown.
into main ducts of the dorsal prostate. So of roughly 52 “dorsolateral” buds in the average vehicle-exposed UGS, about 48 will develop into main ducts (42 in the dorsal prostate and 6 in the lateral prostate). Overall, approximately 58 of the 65 or so prostatic epithelial buds present before birth ultimately develop into main prostatic ducts.

**Effects of AhR Null Mutation on Prostatic Epithelial Bud Formation**

We have reported that AhR null mutation causes modest reductions in absolute and relative dorsolateral prostate and anterior prostate weights in vehicle-exposed C57BL/6 mice but has no detectable effect on the ventral prostate (Lin et al., 2002a). In the present study, we found no differences in the timing of ventral, dorsal, lateral, or anterior bud appearance between wild-type and Ahr−/− fetuses, or in the physical appearance, positioning, or number of these buds. These observations demonstrate that the AhR is not necessary for the process of prostatic epithelial bud formation to occur. Whether the modest reductions seen in dorsolateral and anterior prostate weights of vehicle-exposed AhRKO mice are due to more subtle effects on bud formation not detectable by SEM, or to mechanisms other than budding, remains to be determined.

**Effects of in Utero TCDD Exposure on Prostatic Epithelial Bud Formation**

In contrast to the effects of AhR null mutation, effects of in utero TCDD exposure on ventral and dorsolateral bud forma-
tion correlate well with the effects of in utero and lactational TCDD exposure on ventral and dorsolateral prostate development. Effects of TCDD on weight, ductal structure, and expression of a prototypical androgen-dependent gene are more severe in the ventral prostate than in the other prostate lobes (Ko et al., 2002; Lin et al., 2002a,b). We have now found that the same maternal dose of TCDD (5 μg/kg) causes a complete inhibition of ventral budding. Although ventral buds begin to appear in control UGS on GD 17, and although each control UGS had eight ventral buds on GD 18, UGS from TCDD-exposed mice still had no ventral buds on GD 19/PND 0. We conclude that the absence of ventral budding from the UGS is the major cause of the severe inhibition of ventral prostate development in TCDD-exposed wild-type mice. Effects of in utero and lactational TCDD exposure on dorsolateral prostate weight and ductal branching morphogenesis are far less severe than its effects on the ventral prostate. In addition, expression of mRNA for prototypical androgen-dependent dorsolateral prostate genes was not significantly inhibited by TCDD, in contrast to a severe inhibition in the ventral prostate (Ko et al., 2002; Lin et al., 2002a,b). Similarly, the inhibition of dorsolateral budding was much less severe than the effect on ventral budding, both in terms of the reduction in bud number on GD 18 and in terms of the delay in their appearance (dorsal and

FIG. 8. Effects of in utero DHT exposure on UGS structure in vehicle- and TCDD-exposed wild-type mice on GD 18.

FIG. 9. Effects of in utero DHT exposure on the numbers of ventral and dorsolateral UGS buds in vehicle- and TCDD-exposed wild-type mice on GD 18. Results are means ± SE, n = 6–16. *Significantly different from the corresponding vehicle-exposed control at p < 0.05.
lateral buds were delayed by about a day, whereas ventral buds never appeared). It therefore appears that the inhibition of dorsal and lateral budding from the UGS is a contributing factor to the effects of TCDD on the dorsolateral prostate seen later in development.

TCDD had no detectable effect on the shape of anterior prostatic buds or on their number on GD 18, but anterior buds were generally smaller in TCDD-exposed than in vehicle-exposed wild-type mice, presumably because TCDD delayed their appearance by about a day. The reduction in anterior bud size may contribute to reductions in absolute and relative anterior prostate weight, androgen-dependent gene expression, and ductal branching morphogenesis seen postnatally in TCDD-exposed wild-type mice (Ko et al., 2002; Lin et al., 2002a,b).

The inhibitory effects of TCDD on ventral, dorsolateral, and anterior bud formation and growth are AhR dependent, as are the inhibitory effects of TCDD on postnatal weight and gene expression in each prostate lobe (Lin et al., 2002a). This further supports the conclusion that the inhibition of prostatic epithelial bud formation is a principal factor responsible for these effects. Whether the region-selective effects of TCDD are due in part to possible differences in AhR distribution within the UGS remains to be determined.

One apparent discrepancy between prostatic epithelial budding and subsequent prostate development in TCDD-exposed mice is that these mice develop a lateral prostate despite having few if any buds on the lateral surfaces of their UGS during fetal development. Yet TCDD-exposed mice typically have more buds on the dorsal surface of their UGS than do control mice. It therefore appears that in utero TCDD exposure causes what would normally be lateral prostatic buds to develop on the dorsal surface of the UGS. These additional buds are presumably the origin of the lateral prostate in TCDD-exposed mice.

The apparent development of lateral buds on the dorsal surface of the UGS, as well as the differential effects of TCDD on ventral, dorsolateral, and anterior bud numbers, suggest that TCDD affects the expression of genes responsible for patterning. Among the candidate genes whose expression may be altered by TCDD are the homeobox genes HoxA-13 and HoxD-13, which are known to play lobe-selective roles in prostate development (Podlasek et al., 1997, 1999). TCDD may also cause region-selective effects on fibroblast growth factor-10 and/or p63, each of which is needed for prostatic epithelial bud formation (Donjacour et al., 2003; Signoretti et al., 2000), or on bone morphogenetic protein 4, which inhibits budding (Lamm et al., 2001). Given the complexities of UGS and prostate development (Marker et al., 2003) and of the AhR signaling pathway through which TCDD acts (Carlson and Perdew, 2002; Gasiewicz and Park, 2003), numerous mechanisms can be postulated to account for the effects of TCDD on prostatic epithelial bud formation. Further research is needed to discriminate among these possibilities.

We previously reported that in utero TCDD exposure inhibits prostatic epithelial bud formation in the Holtzman rat (Roman et al., 1998). Dorsal, lateral, and ventral bud numbers were reduced in TCDD-exposed fetuses by 25, 21, and 33%, respectively, on GD 20, and the dorsal budding line was shortened by 27%. Total cross sectional area of buds in each region (equivalent to a weight measurement) was not significantly affected, however. Effects of TCDD on prostatic epithelial budding in rats were most severe in male fetuses that were adjacent, within the uterus, to two females (Timms et al., 2002). We did not specifically investigate whether effects of TCDD on mouse UGS were affected by intrauterine position, but any such effects, if present, are likely to be minor at the TCDD dose used. The primary differences between effects of in utero TCDD exposure on prostatic epithelial bud formation in the rat and mouse are that the effects of TCDD are far more region-specific in the mouse, and that the inhibition of ventral budding in the mouse is far more severe than in the rat.

Inhibited Prostatic Epithelial Bud Formation in TCDD-Exposed Fetuses Is Not Due to Insufficient DHT

Because prostatic epithelial bud formation is androgen-dependent (Cunha et al., 1987), and because TCDD inhibits a number of androgen-dependent processes (Theobald et al., 2003), we tested the hypothesis that the inhibition of prostatic epithelial bud formation caused by TCDD could be prevented by supplying exogenous DHT to the fetuses. Although sufficient DHT was implanted in pregnant mice to greatly elevate circulating maternal DHT concentrations and to strongly masculinize female fetuses, DHT conferred no protection against the inhibitory effects of TCDD on prostatic epithelial bud formation. While the results of this experiment cannot exclude the possibility that in utero TCDD exposure severely inhibits responsiveness of the UGS to androgenic stimulation, it demonstrates that the inhibition of budding is not due to a possible reduction in DHT availability.

Directions for Future Research

Results presented in this manuscript provide the first direct evidence that TCDD inhibits prostate development in the mouse, at least in part, by inhibiting the initial morphological stage of its ontogeny. The finding that in utero TCDD exposure inhibits ventral and dorsolateral budding confirms and extends our previous findings that the critical windows for inhibitory effects of TCDD on prostate development in the mouse includes or is restricted to prenatal stages (Lin et al., 2002b). In light of these results, we are now focusing our research on the molecular control of prostatic bud formation and on the mechanisms by which this process is disrupted by TCDD.

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