Lack of Expression of EGF and TGF-α in the Fetal Mouse Alters Formation of Prostatic Epithelial Buds and Influences the Response to TCDD

Barbara D. Abbott, Tien-Min Lin, Nathan T. Rasmussen, Ralph M. Albrecht, Judith E. Schmid, and Richard E. Peterson

Received August 2, 2003; accepted September 3, 2003

In utero, 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) exposure causes abnormal ventral, dorsolateral, and anterior prostate development in C57BL/6J mice. Androgens, mesenchymal-epithelial interactions, and growth factor expression all have roles in initiating and regulating development and growth of the prostate. Epidermal growth factor (EGF) and transforming growth factor alpha (TGF-α), both of which bind the EGF receptor (EGFR), are expressed in human and rodent developing prostate. This study examines the influence of null expression of EGF and/or TGF-α on prostatic bud development and on the ability of TCDD to inhibit prostatic budding. Growth factor knockout (-/-) and wild-type (WT) mice were exposed either to vehicle or to TCDD (0, 0.2, 1, 5, 10, 50, 100, or 150 μg/kg) on gestation day (GD) 12. The number of anterior, dorsal, and lateral prostatic buds (ADLB) and ventral buds (VB) were counted on GD 17.5. Control WT and EGF (-/-) fetuses had similar numbers of ADLB and VB. In control TGF-α (-/-) fetuses, the number of ADLBs was higher relative to the C57BL/6J. Control EGF + TGF-α (-/-) had poor bud outgrowth, especially in the ADL region. TCDD induced a dose-related decrease in bud formation in all strains with the formation of VBs being more sensitive than ADLBs. The severity of the response depended on growth factor expression, with the most severe effects on VBs in the EGF (-/-) and on ADLBs in the EGF + TGF-α (-/-) fetuses. TGF-α (-/-) and C57BL/6J fetuses responded to TCDD similarly. In conclusion, EGF and TGF-α expression are important for the formation of ADLBs and VBs, and expression of EGF and TGF-α affects the ability of TCDD to inhibit prostatic bud formation in a region-specific manner.

Key Words: prostatic bud; TCDD; urogenital sinus; prostate development.

Portions of this research were presented at the 41st annual meeting of the Society of Toxicology, Nashville, TN, March 17–21, 2002. This article is Contribution 349 from the Molecular and Environmental Toxicology Center, University of Wisconsin, Madison, WI 53705.

Development of the prostate in the mouse is inhibited by in utero and lactational exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) (Lin et al., 2002a,b; Sommer, 1997; Theobald and Peterson, 1997). The weights of prostatic lobes are reduced in C57BL/6J mice after exposure to a single 5 μg/kg maternal dose of TCDD on gestational day (GD) 13 (Ko et al., 2002; Lin et al., 2002b). This weight reduction correlates with an inhibition of outgrowth of the prostatic epithelial buds that develop from the urogenital sinus (UGS), and the severity of this response varies regionally in the UGS with the strongest effect in the ventral region (Ko and Peterson, 2003; Lin et al., in press). Both in utero and lactational exposure of the male rat to TCDD decreases ventral prostate epithelial cell proliferation and disrupts differentiation and organization of the ducts (Roman et al., 1998). There is evidence that TCDD acts directly on the UGS to inhibit prostatic bud formation (Upton et al., 2002) and that androgen signaling is not inhibited (Ko and Peterson, 2003). The effects of TCDD on prostatic bud development do not appear to be androgen-dependent, as treatment with 5α-dihydrotestosterone (DHT) fails to protect against the inhibition of bud formation from the UGS (Lin et al., in press).

Prostate development requires regulation through a number of hormonal, cellular, and molecular pathways (Marker et al., 2003). Epithelial-mesenchymal interactions are essential components of this regulatory battery (Cunha et al., 1980; Takeda et al., 1986; Timms et al., 1995). The mesenchyme of the UGS induces outgrowth and ductal morphogenesis and regulates proliferation of the epithelial cells (Cunha et al., 1992; Tanji et al., 2001). Growth factors regulate cellular proliferation, differentiation, and death, and there are numerous studies implicating growth factors as mediators of epithelial-mesenchymal interactions. EGF, TGF-α, TGF-β, insulin-like growth factors I and II, hepatocyte growth factor (HGF), and keratinocyte growth factor (KGF) are known to either stimulate or inhibit growth of the prostate (Tanji et al., 2001).

EGF and TGF-α mRNA are detected in human fetal prostatic tissue and these growth factor proteins localize to basal epithelial cells (Dahiya et al., 1996; Leav et al., 1998). Raghow
et al. (1999) detected TGF-α protein only in the mesenchyme of the 9.5 to 11.5 week old human fetus. EGF and TGF-α also exhibit localized expression patterns in the adult human prostate (Adam et al., 1999; Cohen et al., 1994; De Miguel et al., 1999). There are several studies that examined expression of EGF and TGF-α mRNA and/or protein in the neonatal rat. Analysis of neonatal and postnatal day (PND) 0 rat ventral prostate grown in organ culture detected cell type–specific expression of keratinocyte growth factor (KGF), KGF receptor, TGF-α, and EGF receptor (Thomson et al., 1997). TGF-α was found to be predominantly epithelial in this study as well as in a study of the PND 5–20 rat prostate in which TGF-α was localized to ductal epithelium of the ventral prostate (Banerjee et al., 1998). In the rat at PND 63, expression of TGF-α was detected immunohistochemically in epithelial cells of the ventral and lateral prostatic ducts but not in the dorsal ducts (Taylor and Ramsdell, 1993). Prostate lobe–specific expression of TGF-α was also reported in the adult rat, where it was detected in the lateral lobe with fewer immunostained cells in the dorsal lobe and no expression in the ventral lobe; however, EGF was detected in all lobes of the adult prostate (Wu et al., 1993). Thus, as has been noted in a number of other developing organ systems, there appears to be specific spatio-temporal expression of growth factors during development and growth of the prostate.

We examined the influence of null expression of EGF and/or TGF-α in the fetal mouse on prostatic bud formation and on the response to TCDD. Knockout mice lacking expression of EGF (-/-), TGF-α (-/-), or both EGF and TGF-α (EGF + TGF-α -/-) were exposed in utero to either vehicle or TCDD and the effects on prostatic bud development were evaluated on GD 17.5. We previously studied the EGF (-/-), TGF-α (-/-), and EGF + TGF-α (-/-) and reported no effect of genotype or background strain on litter size, gender ratio, pup body weight, and survival of fetuses in utero (Bryant et al., 2001). In that study, exposure to 24 μg TCDD/kg increased the maternal and fetal liver weights in all strains and genotypes, both absolute and relative to body weight. The EGF (-/-), TGF-α (-/-), and EGF + TGF-α (-/-) strains were also examined across a range of doses (0.2 to 150 μg TCDD/kg), and there was no increase in maternal or fetal deaths and no effects on maternal body weight at any of the doses (Abbott et al., 2003). This dosing regimen was applied to the present study for evaluation of the effects of TCDD on prostatic bud development. Lack of expression of EGF and/or TGF-α influenced the development of the prostatic buds and affected the responsiveness to TCDD.

**MATERIALS AND METHODS**

**Animals.** Colonies of EGF (-/-), TGF-α (-/-), EGF + TGF-α (-/-), and wild-type (WT) mice were maintained in the animal facility at the U.S. Environmental Protection Agency, Research Triangle Park, NC. The EGF (-/-) and EGF + TGF-α (-/-) mice were originally obtained from Dr. David Lee at the University of North Carolina (Chapel Hill, NC). These mice were derived from 129 and C57BL/6J strains (Lee et al., 1995; Luetteke et al., 1993, 1999) and were of mixed genetic background; as such, appropriate wild-type (WT) mice with 129 × C57BL/6J background were also provided by Dr. Lee’s laboratory. The TGF-α (-/-) mice (Tgfatm1Ard), developed by Dr. Dunn at the Ludwig Institute for Cancer Research (Mann et al., 1993), were obtained from Jackson Laboratory (Bar Harbor, ME). The TGF-α (-/-) were backcrossed over 10 generations into the C57BL/6J background by Jackson Laboratory. Thus, timed-pregnant C57BL/6J mice were obtained from Jackson Laboratory as the appropriate wild type for comparison to the TGF-α (-/-). Food (Agway rat, mouse, and hamster 3000; Agway, Inc., Syracuse, NY) and distilled water were provided ad libitum. All animals were housed under controlled conditions of temperature (22 ± 2°C), 50–60% humidity, and light (12/12 h light/dark cycle, 6:00 AM to 6:00 PM) and treated as approved by NHEERL IACUC. All of the homozygous knockout strains of mice were normal and maintained good health throughout the study. The EGF (-/-) did not display any observable phenotype and the EGF + TGF-α (-/-) and TGF-α (-/-) displayed a pronounced waviness in the coat and whiskers, consistent with the reported phenotype associated with knockout of TGF-α (Lee et al., 1995; Luetteke et al., 1993, 1999; Mann et al., 1993).

At the University of Wisconsin, C57BL/6J mice were from a C57BL/6J breeding colony established with mice purchased from Jackson Laboratory. These mice were housed in clear plastic cages with heat-treated aspen bedding in rooms that were kept at 24 ± 1°C and 35 ± 4% relative humidity and lighted from 0600 to 1800 h. Food (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.

Male and female mice of the same genetic background were housed together overnight. Females were checked for vaginal plugs and weighed the next morning, which was designated as gestation day (GD) 0; plug-positive females were weighed.

**Treatments.** Pregnant females were weighed on GD 12 and dosed by gavage with 0, 0.2, 1, 5, 10, 50, 100, or 150 μg TCDD/kg body weight at a dose volume of 5 ml/kg. The number of pregnant females (litters) for each dose and genotype are presented in Tables 1 and 2. The assignment of dose groups by genotype was based on results from our previous study (Abbott et al., 2003) in which genetic background was shown to affect responsiveness to TCDD. Responsiveness to TCDD was evaluated in that study using EROD assays of adult liver microsomes, which showed that the WT, EGF (-/-), and EGF + TGF-α (-/-) strains were less responsive than the C57BL/6J and TGF-α (-/-) strains. Based on the EROD outcomes, it was concluded that the WT, EGF (-/-), and EGF + TGF-α (-/-) strains express a low affinity isofrom of the AhR, while the C57BL/6J and TGF-α (-/-) strains express a high affinity AhR allele.

**Necropsies and analytical procedures.** On GD 17.5, pregnant females were anesthetized by CO2 inhalation and killed by cervical dislocation. The fetuses were removed from the uterus and placed in petri dishes containing ice-cold phosphate-buffered saline. The fetuses were decapitated and viscera removed from the abdominal cavity. Male fetuses were identified by gonadal inspection and these fetuses were bisected, separating the upper and lower abdominal regions with a scalpel just anterior to the bladder. The lower half of the fetal body, which included the intact urogenital sinus (UGS), was merged in ice-cold phosphate-buffered saline. The fetuses were decapitated and viscera removed from the abdominal cavity. Male fetuses were identified by gonadal inspection and these fetuses were bisected, separating the upper and lower abdominal regions with a scalpel just anterior to the bladder. The lower half of the fetal body, which included the intact urogenital sinus (UGS), was submerged in ice-cold Hank’s balanced salt solution (HBSS, Sigma Chemical Co., St. Louis, MO), supplemented with 5% charcoal/dextran-stripped FBS. All of the male fetuses from each litter were prepared as described. After each necropsy, the tissues were shipped overnight in a cold-pack, insulated shipping container to the University of Wisconsin (Madison, WI). The C57BL/6J females that were bred, dosed, and necropsied at the University of Wisconsin were subjected to the same treatment and necropsy procedures as described for the mice at the EPA, except that at necropsy the processing of the tissues continued without the overnight delay. On arrival (or after bisecting the fetal lower body for tissues prepared at the University of Wisconsin), the UGS complexes were removed and subjected to limited trypsin digestion to separate epithelium from mesenchyme by procedures similar to those described by Cunha and Donjacour (1987a,b). Briefly, UGSs were incubated in calcium- and magnesium-free HBSS containing 1% trypsin (Gibco BRL, Grand Island,
TABLE 1
The Effect of TCDD on Number of Prostatic Buds in Different Regions of the Urogenital Sinus in WT, EGF (-/-), and EGF + TGF-α (-/-) Mouse Fetuses on Gestation Day 17.5

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Treatment</th>
<th>n</th>
<th>Anterior-dorsolateral buds (mean ± SEM)</th>
<th>Ventral buds (mean ± SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT</td>
<td>0</td>
<td>5</td>
<td>59.8 ± 2.4</td>
<td>8.8 ± 0.4</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>4</td>
<td>63.8 ± 1.4</td>
<td>9.4 ± 1.0</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>6</td>
<td>32.8 ± 1.8***</td>
<td>7.3 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>6</td>
<td>37.9 ± 1.7***</td>
<td>4.2 ± 1.0***</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>3</td>
<td>37.0 ± 4.7***</td>
<td>3.7 ± 0.9***</td>
</tr>
<tr>
<td></td>
<td>150</td>
<td>5</td>
<td>31.8 ± 3.3***</td>
<td>1.0 ± 0.6***</td>
</tr>
<tr>
<td>EGF (-/-)</td>
<td>0</td>
<td>5</td>
<td>54.8 ± 4.3</td>
<td>9.1 ± 1.1</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>4</td>
<td>69.5 ± 7.4</td>
<td>9.4 ± 1.2</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>6</td>
<td>43.8 ± 4.0</td>
<td>2.6 ± 1.0***</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>4</td>
<td>38.6 ± 2.3*</td>
<td>0.0 ± 0.0***</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>3</td>
<td>15.7 ± 8.8***</td>
<td>0.0 ± 0.0***</td>
</tr>
<tr>
<td></td>
<td>150</td>
<td>1</td>
<td>15.0 ± 0.0**</td>
<td>0.0 ± 0.0***</td>
</tr>
<tr>
<td>EGF + TGF-α (-/-)</td>
<td>0</td>
<td>4</td>
<td>34.4 ± 5.0†††</td>
<td>7.1 ± 0.4</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>5</td>
<td>29.1 ± 2.0</td>
<td>7.1 ± 0.6</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>3</td>
<td>28.9 ± 3.0</td>
<td>6.7 ± 0.3</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>6</td>
<td>25.4 ± 2.6*</td>
<td>0.8 ± 0.6***</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>5</td>
<td>11.3 ± 2.6***</td>
<td>0.0 ± 0.0***</td>
</tr>
<tr>
<td></td>
<td>150</td>
<td>5</td>
<td>10.7 ± 2.3***</td>
<td>0.0 ± 0.0***</td>
</tr>
</tbody>
</table>

*TCDD dose in μg/kg maternal body weight.
†SEM, standard error of mean.
‡p < 0.05, ††p < 0.01, †††p < 0.001 compared to control, within genotype.
Dose-related trend p < 0.001, ADLBs and VBs, all genotypes. ‡p < 0.01 compared to EGF -/- control, ††p < 0.01 compared to WT control. †††p < 0.05, ‡p < 0.001 compared to receiving same dose. Genotype interactions (p < 0.001); for ADLBs, WT and EGF + TGF-α; EGF and EGF + TGF-α; for VBs, WT and EGF; WT and EGF + TGF-α. Antigen and dose interactions: for ADLBs, WT and EGF (p < 0.01); WT and EGF + TGF-α (p < 0.001); EGF and EGF + TGF-α (p < 0.01); for VBs, WT and EGF (p < 0.05); EGF and EGF + TGF-α (p < 0.01).

RESULTS

The removal of the UGS mesenchyme by enzymatic treatment and subsequent viewing of the UGS epithelia with scanning electron microscopy (SEM) provide a method for visualizing and quantifying the development of the prostatic epithelial buds (Lin et al., 2003). Quantitative analysis of budding patterns required at least four images of each fixed UGS epithelium. Figure 1A shows the lateral view of representative UGS epithelial structures of a WT fetus. As described in Lin et al. (2003), the GD 17.5 ventral buds were shaped like elongated cylinders that often had a slightly enlarged distal end. Dorsal buds are somewhat shorter than ventral buds and are cylindrical at the base but usually widen into a bulbous top. Anterior buds are similar in shape to dorsal buds but are typically much larger. Lateral buds also resemble dorsal buds but are typically longer. The remaining prostatic epithelial buds are located on the lateral surfaces of the UGS. These buds 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. By GD 18, the number of ADLBs and VBs have each reached their maximum (Lin et al., 2003).

TABLE 2
The Effect of TCDD on Number of Prostatic Buds in Different Regions of the Urogenital Sinus in C57BL/6J and TGF-α (-/-) Mouse Fetuses on Gestation Day 17.5

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Treatment</th>
<th>n</th>
<th>Anterior-dorsolateral buds (mean ± SEM)</th>
<th>Ventral buds (mean ± SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C57BL/6J</td>
<td>0</td>
<td>7</td>
<td>42.5 ± 2.5</td>
<td>7.0 ± 0.3</td>
</tr>
<tr>
<td></td>
<td>0.2</td>
<td>7</td>
<td>36.0 ± 1.4</td>
<td>5.0 ± 0.3</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>6</td>
<td>32.0 ± 3.7**</td>
<td>2.6 ± 1.0***</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>5</td>
<td>24.8 ± 2.8***</td>
<td>0.0 ± 0.0***</td>
</tr>
<tr>
<td>TGF-α (-/-)</td>
<td>0</td>
<td>4</td>
<td>52.5 ± 3.2</td>
<td>7.3 ± 0.5</td>
</tr>
<tr>
<td></td>
<td>0.2</td>
<td>4</td>
<td>53.8 ± 2.3</td>
<td>7.5 ± 1.3</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>4</td>
<td>40.6 ± 2.5*</td>
<td>1.9 ± 1.1***</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>5</td>
<td>32.6 ± 3.0***</td>
<td>0.0 ± 0.0***</td>
</tr>
</tbody>
</table>

*TCDD dose in μg/kg maternal body weight.
†SEM, standard error of mean.
‡p < 0.05, ††p < 0.01, †††p < 0.001 compared to control. Dose-related trend p < 0.001, ADLBs and VBs, both genotypes. Genotype interaction for ADLBs, p < 0.001.
In all genotypes, there were significant dose-related decreases in the number of ADLBs and VBs in response to TCDD ($p < 0.001$). The absence of EGF and/or TGF-α expression significantly affected the responsiveness to TCDD, and the responses of the different genotypes were not identical. This was detected in analysis of genotype by TCDD dose interactions, which were significant for ADLB effects: WT versus EGF (-/-), $p < 0.01$; WT versus EGF + TGF-α (-/-), $p < 0.001$; and EGF (-/-) versus EGF + TGF-α (-/-), $p < 0.01$. For VB effects: WT versus EGF (-/-), $p < 0.05$; and EGF + TGF-α (-/-) versus EGF (-/-), $p < 0.01$. Genotype interaction was also significant for VB: WT versus EGF (-/-), $p < 0.001$; and WT versus EGF + TGF-α (-/-), $p < 0.001$.

In the WT mice, there was a significant decrease in ADLB numbers after exposure to 10 μg TCDD/kg body weight; however, the VB numbers were only affected significantly at 50 μg TCDD/kg or higher. The effects on ADLBs appeared to plateau at 50 μg TCDD/kg when the mean values were examined; however, as shown in Figure 1, fetuses exposed to 150 μg TCDD/kg exhibited more severe effects than observed in the lower exposures. There was a significant TCDD dose-related trend for effects on VBs. The numbers of these buds were observed to be less as TCDD exposures increased in the representative UGS images of prostatic bud formation (Figs 1B–1F). The fetuses exposed at 100 and 150 μg TCDD (Figs 1E and 1F) exhibited fewer and smaller dorsal buds and appeared not to have VBs. Also, as shown graphically in Figure 2A, responsiveness of ADLBs to TCDD appeared to plateau at a dose of 10 μg/kg in the WT group, as there was no further decrease in the ADLBs with increasing TCDD dose. This is in contrast to the response of the ventral UGS region in which the VBs continued to decrease across all TCDD doses. The UGS shown in Figures 1D–1F exhibit few or no VBs.

Responses of EGF (-/-) and EGF + TGF-α (-/-) fetuses also showed significant TCDD dose-related decreases in ADLBs and VBs (Figs 2A and 2B). In the absence of EGF expression, the trend for decreasing ADLBs did not appear to plateau (Fig. 2A), as appeared to occur in the WT mice. The EGF (-/-) and EGF + TGF-α (-/-) fetuses appeared more sensitive to the inhibitory effects of TCDD on ADLB bud numbers than the WT, particularly at the higher TCDD doses. The EGF (-/-) and EGF + TGF-α (-/-) fetuses were clearly more sensitive than the WT to TCDD reduction in VB at all doses (Fig. 2B). In the anterior-dorsolateral regions, the EGF (-/-) and EGF + TGF-α (-/-) fetuses showed significant ADLB reductions relative to control at 50 μg/kg TCDD and higher. In contrast, the reductions in ADLB bud number in WT were significant after exposure to 10 μg/kg TCDD (Table 1). However, in the ventral region, the EGF (-/-) appeared to be more sensitive than WT or EGF + TGF-α (-/-) to the effects of TCDD (Fig. 2B), with significant decreases after exposure to 10 μg TCDD/kg (compared to significant responses at 50 μg/kg for WT and EGF + TGF-α (-/-); Table 1). The main effects model and regression analysis indicated a significant effect of genotype in the com-

![FIG. 1. Scanning electron micrographs show the GD 17.5 urogenital sinus (UGS) epithelium from control and TCDD-exposed wild-type (WT) fetuses. (A) The UGS of a control fetus exhibited dorsal, ventral, lateral, and anterior buds. (B–F) TCDD-exposed fetuses have fewer and smaller buds and the response was more severe as the GD 12 exposure increased from 1 to 150 μg/kg of TCDD (B–F, respectively). A 10 μg/kg dose reduced bud size and numbers in anterior, dorsal, and lateral regions, but reduction of the ventral buds was seen only after exposure to 50 μg/kg TCDD or higher. Ab, anterior bud; bn, bladder neck; db, dorsal bud; lb, lateral bud; vb, ventral bud; u, urethra; *, a region with few or no buds. Scale bar = 150 μm.](https://academic.oup.com/toxsci/article-abstract/76/2/427/1686043?redirectedfrom=fulltext)
The effects of increasing TCDD dose on the number and size of prostatic buds in EGF (-/-) and EGF + TGF-α (-/-) UGS are shown for representative fetuses in Figs 3 and 4. In the EGF (-/-) fetus, TCDD exposure reduced the number and size of the buds and the response was more severe as the dose increased from 1 to 150 μg TCDD/kg (Figs 3B–3F). Although the trend for this effect was present across the doses, significance relative to control was only present at exposures of 50 μg TCDD/kg or higher. Ventral buds were strongly reduced at 10 μg TCDD/kg and frequently no buds were observed in that region (Fig. 3C). At exposures of 50 μg TCDD/kg or higher, few buds were seen on the EGF (-/-) UGS. In the EGF + TGF-α (-/-) fetus, the decrease in ADLBs and VBs was apparent after a dose of 1 μg TCDD/kg (Fig. 4B), and few or no buds were seen at a dose of 50 μg/kg or higher (Figs 4D–4F). In the EGF + TGF-α (-/-), the effects of TCDD on VB and ADLB were significant at doses of 50 μg TCDD/kg or higher.

FIG. 2. The TCDD dose response in WT, EGF (-/-), and EGF + TGF-α (-/-) GD 17.5 fetuses is shown as the mean numbers of (A) anterior-dorsolateral buds (ADLBs) and (B) ventral buds (VBS) in the GD 17.5 UGS after exposure to TCDD on GD 12. In all genotypes, there was a significant TCDD dose–related trend for decreased bud numbers in both regions. The response of ADLBs in the WT appeared to plateau at higher TCDD exposures. Across both regions of the UGS, the EGF (-/-) and EGF + TGF-α (-/-) fetuses were significantly more sensitive than the WT to TCDD-induced bud reduction. In the anterior-dorsolateral region, the fetuses lacking both EGF and TGF-α were most severely affected. VB formation and growth was most affected in fetuses lacking only EGF expression.

FIG. 3. Scanning electron micrographs of the GD 17.5 EGF (-/-) fetus show the outgrowth of prostatic buds from the urogenital sinus (UGS) epithelium. (A) Control fetuses lacking EGF expression had prostatic buds in the ventral, anterior, lateral, and dorsal regions. (B–F) TCDD exposure reduced the numbers and size of the buds, and the response was more severe as the GD 12 exposure increased from 1 to 150 μg/kg (B–F, respectively). Ventral buds were strongly reduced at the 10 μg/kg dose and no buds could be seen in that region for the fetus shown in (C). At exposures of 50 μg/kg or higher, few buds were observed in the other regions as well. Ab, anterior bud; bn, bladder neck; db, dorsal bud; lb, lateral bud; vb, ventral bud; u, urethra; * (black or white), a region with few or no buds. Scale bar = 150 μm.
Relative to WT (Fig. 1A) and EGF (-/-) (Fig. 3A), the EGF/TGF-α (-/-) control (Fig. 4A) had fewer and less developed prostatic buds. It would appear that in the mixed genetic background found in WT, EGF (-/-), and EGF/TGF-α (-/-) fetuses, expression of TGF-α, or both EGF and TGF-α, is important for outgrowth and formation of the prostatic buds.

Effects of in Utero Exposure to TCDD on Prostatic Bud Formation in C57BL/6J and TGF-α (-/-) Fetuses

The C57BL/6J and TGF-α (-/-) fetuses responded to lower doses of TCDD relative to the WT, EGF (-/-), and EGF + TGF-α (-/-) genotypes (Table 2). This is attributed to differences in the AhR alleles associated with the background genetics of these strains. The TGF-α (-/-) fetuses are on the C57 background, which confers a high affinity AhR. The effects of TCDD on prostatic bud development are detectable at 0.2 μg/kg TCDD (Figs 5B and 6B) and are significant for decreasing the number of ADLBs and VBs at 1 μg/kg (Table 2). The C57BL/6J and TGF-α (-/-) fetuses exhibited similar, significant (p < 0.001) TCDD dose–response trends for decreased numbers of ADLBs and VBs (Figs 7A and 7B), and there was not a significant genotype by dose interaction for either prostatic region in these fetuses. However, in the anterior-dorsolateral region, there was a significant (p < 0.001) genotype effect and the TGF-α (-/-) fetuses had more ADLBs than the C57BL/6J strain across all groups (Table 2). This was not the case in the ventral region, as the VB numbers were indistinguishable for these genotypes across all doses. These region-specific differences are apparent in the representative UGS images of the C57BL/6J (Figs 5A–5D) and TGF-α (-/-) (Figs 6A–6D). In the fetuses exposed to 1 or 5 μg/kg TCDD (Figs 5, 6C, and 6D), no VBs can be seen and only a few ADLBs are apparent in the C57BL/6J fetuses.
DISCUSSION

The expression of EGF and TGF-α influences development of prostatic buds from the UGS and the sensitivity of the UGS to inhibition of prostatic bud formation following in utero exposure to TCDD. The availability of gene knockout strains of mice, which do not express EGF, TGF-α, and both EGF + TGF-α, provided an opportunity to evaluate the influence of EGF and TGF-α on outgrowth of prostatic buds in the anterior-dorsolateral and ventral regions of the UGS. This study revealed that development and outgrowth of the prostatic buds is influenced by expression of both EGF and TGF-α. In the fetuses that expressed EGF in the absence of TGF-α [as occurs in the TGF-α (-/-)], ADLB formation was stimulated relative to the vehicle-exposed controls. This stimulation did not happen when TGF-α was expressed and EGF was the missing growth factor [as is the case for the EGF (-/-) and the EGF + TGF-α (-/-)]. Also, in the absence of both EGF and TGF-α, prostatic bud formation was inhibited. Relative to wild-type fetuses or to fetuses that express at least one of the growth factors, these double-null fetuses have poor prostatic bud outgrowth, especially in the ADL region.

One possible interpretation of these observations is that balanced expression of EGF and TGF-α regulates bud outgrowth and that at least one of these growth factors needs to be expressed. The role of each growth factor in influencing bud numbers may be different, as TGF-α expressed in the absence of EGF was able to produce appropriate numbers of buds in all regions, while EGF expressed in the absence of TGF-α appeared to overstimulate ADLB outgrowth. It is also possible that TGF-α acts as a negative regulator or suppressor of ADLB development and that could also explain the increased numbers of ADLB when TGF-α is not expressed. From these data, it was not possible to definitively identify the activity that results in more ADLB, stimulation by EGF, or negative regulation by TGF-α. It may also be hypothesized that UGS region–specific patterns of EGF and TGF-α expression are required to provide balanced growth factor expression across the UGS.

FIG. 6. (A) The UGS of control fetuses that lack TGF-α expression showed prostatic bud development in all UGS regions. (B) Exposure to 0.2 μg/kg TCDD did not appear to affect bud outgrowth. (C and D) After exposure to 1 or 5 μg/kg TCDD (C and D, respectively), no ventral buds could be observed in these fetuses and the size and numbers of buds in the other regions were reduced. Bn, bladder neck; db, dorsal bud; vb, ventral bud; u, urethra; *, a region with few or no buds. Scale bar = 150 μm.

FIG. 7. In the C57BL/6J and the TGF-α (-/-) fetuses, a significant TCDD dose–related trend for decreased numbers of prostatic buds was observed for (A) ADLBs and (B) VBs. The genotypes exhibited similar trends for decreasing numbers of buds in both regions. The TGF-α (-/-) UGS had more ADLBs than the C57BL/6J across all TCDD dose groups. VB outgrowth and response to TCDD was indistinguishable for C57BL/6J and TGF-α (-/-) fetuses.
The expression of these growth factors also affects the sensitivity of the fetal response to TCDD. The influences of each growth factor on the response to TCDD differ and may reflect divergent roles in regulation of bud outgrowth. The effects of TCDD were most severe in the double-null fetuses that had neither EGF nor TGF-α expression. The fetuses without TGF-α expression and the C57BL/6J fetuses responded to TCDD similarly, suggesting that TGF-α is not playing a critical or determinant role in the response to TCDD. However, the increased severity of VB response in the EGF + TGF-α (-/-) relative to the EGF (-/-), i.e., the significant genotype and dose interaction, suggests that TGF-α as well as EGF mediate the response. Although fetuses with EGF expression respond to TCDD with dose-related decreases in prostatic bud outgrowth, the response is clearly more severe in the absence of EGF, particularly in the ventral region. The modulation of the response to TCDD by EGF may be through a mechanism involving upregulation of the growth factor. Increased EGF expression might stimulate prostatic bud outgrowth sufficiently to counteract the inhibitory effects of TCDD. This could explain the more severe responses of fetuses lacking EGF expression, since those compensatory mechanisms would be totally absent in EGF (-/-) and EGF + TGF-α (-/-) fetuses.

A balanced expression of the growth factors may be critical to regulate the development of the prostate, and disruption of that balance by TCDD may be a major factor in abnormal morphogenesis. As stated above, some information is available regarding the expression of EGF and TGF-α during later stages of prostate development but no information could be found in the literature describing patterns of EGF and TGF-α expression during bud formation. Also, there are no reports of the specific effects of TCDD on that expression. However, TCDD is known to alter the expression of these growth factors in the palate and developing urinary tract (Abbott and Birnbaum, 1990a,b; Bryant et al., 1997). Both EGF and TGF-α protein and mRNA are upregulated in response to TCDD in the embryonic palatal epithelium and EGF expression is increased in ureteric epithelial cells. In the urinary tract, as in the prostatic buds, expression of TGF-α in the absence of EGF provided an enhanced response to TCDD (Bryant et al., 2001). In the developing urinary tract, TCDD produced hydronephrosis by stimulating proliferation of the ureteric epithelial cells with a resulting occlusion of the ureteric lumen (Abbott et al., 1987). EGF (-/-) fetuses exhibited an increased incidence of hydronephrosis in response to TCDD (Bryant et al., 2001). In ureteric epithelial cells, both EGF and TGF-α are expressed during formation of the urinary tract. In response to TCDD, EGF but not TGF-α expression was increased, and it was hypothesized that this imbalance in expression of these growth factors plays a role in the hyperplasia of the epithelial cells (Bryant et al., 1997).

In contrast to the increased sensitivity of the UGS and urinary tract in the absence of EGF expression, in the developing palate the absence of EGF expression reduced the incidence of cleft palate (Abbott et al., 2003). In the palate, the predominant ligand expressed is EGF and exposure to TCDD has been shown to increase expression of that growth factor (Abbott and Birnbaum, 1990b). The induction of cleft palate by TCDD is attributed to increased expression of EGF, which correlates with hyperplasia of the medial epithelial cells and consequent failure of the palatal shelves to fuse. Thus, in the absence of EGF [in either the EGF (-/-) or EGF + TGF-α (-/-)], this response was not produced and the fetuses did not exhibit cleft palate at exposures less than 100 µg TCDD/kg. The differential responses reported in the palate, urinary tract, and in this study for the UGS could be due to different spatial and temporal expression patterns of EGF and TGF-α in these tissues. The evidence in the palate and urinary tract supports the hypothesis that the expression patterns during development and the specific alterations in expression produced by TCDD can be instructive concerning the outcome in the target organ.

Detailed information is lacking regarding the expression patterns of EGF and TGF-α in the UGS of the prenatal rodent, and no reports were found that describe specific effects of TCDD treatment on these expression patterns. However, these growth factors are expressed in the fetal human prostate and during development of the prostate postnatally in rodent and human. The in vitro experiments using ventral prostatic cells or neonatal prostatic organ cultures suggest a complex interplay of EGF and TGF-α in regulating growth of the prostate and influencing outgrowth of the prostatic buds. In isolated prismatic epithelial and stromal cells derived from 20-day-old rats, Itoh et al. (1998) reported epithelial expression of TGF-α and EGFR with region-specific expression. This study also reported expression of TGF-α and EGFR mRNA in both epithelial and stromal cells; it examined the interplay of these growth factors and found that exposure to EGF stimulated expression of TGF-α in stromal but not epithelial cells and that proliferation of both cell types was stimulated by EGF. Interestingly, an antibody to inhibit TGF-α activity significantly decreased proliferation in response to testosterone in both cell types.

Numerous in vitro models for cultured rat ventral prostate report stimulated growth of EGF and TGF-α expression in the absence of androgens in the medium. This implies that a complex interplay of growth factor signals is important in maintaining stromal-epithelial interactions that are involved in regulating ductal development of the various lobes of the prostate. Obviously, the complexity of these mesenchymal-epithelial cell interactions, which are integral to formation of buds and subsequent branching, are likely to require expression of regulatory molecules and signaling pathways in addition to the EGFR pathway. Sonic hedgehog, a stimulatory factor, is produced in the UGS, and the homeobox genes Hoxa-13 and
Hoxd-13 play lobe-selective roles in prostate development (Podlasek et al., 1997, 1999). Prostatic epithelial bud formation also requires expression of p63 and fibroblast growth factor-10 (FGF-10; Donjacour et al., 2003; Lamm et al., 2001; Signoret et al., 2000). Several growth factors, including FGF, KGF, and HGF, are known to regulate prostatic growth through a mesenchymal paracrine mechanism (Story, 1995; Sugimura et al., 1996; Thomson, 2001; Thomson and Cunha, 1999).

In summary, this study provides evidence for the importance of EGF and TGF-α in prostate development. The results suggest that both EGF and TGF-α are needed for the formation of the prostatic buds and that their role(s) may differ by region. The differing outcomes in specific regions of the UGS and in the prostatic buds and that their role(s) may differ by region. The differing outcomes in specific regions of the UGS and in EGF (-/-) and TGF-α (-/-) fetuses suggest that UGS region-specific expression patterns of these growth factors may influence the response to TCDD and that a disruption of balanced, UGS region-specific expression could provide a key to understanding the response of the developing prostate to TCDD.

ACKNOWLEDGMENTS

The information in this document has been funded wholly (or in part) by the U.S. Environmental Protection Agency. It has been subjected to review by the Agency, nor does mention of trade names or commercial products constitute endorsement or recommendation for use. Portions of this work were supported by National Institutes of Health grant ES01332 to R. E. P. and by National Institute of Environmental Health Sciences Center grant P30 ES09090 to the EHS Center for Developmental and Molecular Toxicology at the University of Wisconsin. The authors appreciate the expert advice provided during the preparation of this manuscript by Robert W. Moore, School of Pharmacy, Molecular and Developmental Toxicology Center, University of Wisconsin.

REFERENCES


