IN UTERO AND LACTATIONAL EXPOSURE OF MALE RATS TO 2,3,7,8-TETRACHLORODIBENZO-*P*-DIOXIN DECREASES ANDROGEN RESPONSIVENESS OF THE DORSOLATERAL PROSTATE WITHOUT INHIBITING DIHYDROTESTOSTERONE FORMATION

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Introduction

In utero and lactational exposure of male rats to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) impairs anterior, dorsolateral, and ventral prostate growth and development in the absence of any reduction of circulating androgen concentrations. The formation of epithelial buds in the urogenital sinus which give rise to the dorsolateral and ventral prostate is inhibited by TCDD in fetal rats and cellular proliferation of the ventral prostate is decreased on postnatal day (PND) 1. Ventral prostates removed from rats exposed to TCDD *in utero* and via lactation are smaller and, androgen receptor expression and prostatic binding protein secretion is decreased at least until PND 32.[2] Histologically, the TCDD-induced impairment in ventral prostate development is reflected by a disorganized and hyperplastic epithelium which contains fewer luminal (secretory) cells, an increased density of basal cells, and a thicker smooth muscle sheath surrounding the ducts.[2] These results suggest that *in utero* and lactational TCDD exposure can decrease androgen responsiveness in the ventral prostate.

In the dorsolateral prostate androgens stimulate probasin secretion which is mediated by luminal epithelial cell androgen receptors.[1] Since 5α-dihydrotestosterone (DHT), the most potent androgen, is produced locally in the prostate from 5α-androstane 3α, 17β-diol (3α-Diol) and testosterone, androgen responsiveness could be decreased by TCDD causing a reduction in androgen receptor expression, an inhibition of DHT formation, and/or an increase in DHT metabolism. These hypotheses were evaluated in dorsolateral prostates obtained from male rats exposed to TCDD *in utero* and via lactation. The results demonstrate a transient inhibition of probasin mRNA expression in dorsolateral prostates removed from TCDD-treated rats and exposed to graded concentrations of testosterone, 3α-Diol, or DHT in organ culture. Alterations in androgen metabolism included an increase in dorsolateral prostate 17β-hydroxysteroid dehydrogenase activity, but 5α-reductase and 3α-hydroxysteroid dehydrogenase activities were unaffected. Preliminary results indicate that *in utero* and lactational TCDD exposure decreased the expression of androgen receptors in the dorsolateral prostate as has also been found in similarly organ cultured ventral prostates.[3]

Materials and Methods

Pregnant Holtzman rats were administered 1 μg TCDD/kg or vehicle on gestational day (GD) 15. Dorsolateral prostates, obtained from male offspring on PNDs 11 and 18, were incubated in the absence of androgens for 24 hours in Dulbecco's Modified Eagle Medium supplemented with charcoal stripped fetal bovine serum. Each culture was then exposed for 48 hours to 10^{-9}, 10^{-8}, or 10^{-7} M testosterone, 3α-Diol, or DHT and harvested on days that coincided with PND 14 and PND 21. The expression of probasin mRNA was determined by northern blot analysis. Steroid metabolism was determined after similar exposure to 10^{-8} M [^{3}H]-testosterone, [^{3}H]-3α-Diol, or [^{3}H]-DHT for 48 hours in organ culture. Radiolabeled steroids in the medium were extracted, combined with non-radiolabeled steroid standards, separated by thin layer chromatography, and quantified by liquid scintillation counting. Dorsolateral prostate 5α-reductase and 3α-hydroxysteroid dehydrogenase activities were determined in male rat offspring exposed *in utero* and via lactation to a single maternal dose of 0.75 μg TCDD/kg administered on GD 15 by the methods used for ventral prostate enzyme activity measurements.[3]

Results and Discussion

Exposure to testosterone, 3α-Diol or DHT *in vitro* stimulated the expression of probasin mRNA in dorsolateral prostates that were isolated from vehicle-exposed male offspring on PNDs 11 and 18 and cultured to PNDs 14 and 21, respectively (Figure 1). In contrast, almost no androgen-dependent probasin mRNA expression occurred in dorsolateral prostates removed from TCDD exposed male offspring on PND 11 and those removed on PND 18 expressed significantly less probasin mRNA than did dorsolateral prostates from vehicle-exposed offspring. Therefore, partial recovery from the TCDD exposure-induced decrease in androgen responsiveness was apparent in dorsolateral prostates cultured to PND 21. Significantly, exposure of the cultures to DHT did not overcome the transient inhibition of androgen responsiveness. This suggests that the effect of TCDD exposure is not secondary to a decrease in the ability of the dorsolateral prostate to form DHT. Essentially similar results were obtained when the effect of *in utero* and lactational TCDD exposure on androgen-stimulated prostate binding protein mRNA and protein formation was examined in rat ventral prostate organ cultures.[3]

Steroid metabolites in the culture medium were examined after the dorsolateral prostates were exposed to [^{3}H]-testosterone, [^{3}H]-DHT, and [^{3}H]-3α-Diol for 48 hours. While both nonpolar and polar radiolabeled metabolites were recovered in the culture medium, similar amounts of [^{3}H]-DHT were recovered from vehicle and TCDD-exposed dorsolateral prostates cultured with [^{3}H]-testosterone or [^{3}H]-3α-Diol (Figure 2). This suggests that 5α-reductase and 3α-hydroxysteroid dehydrogenase activities were not affected by *in utero* and lactational TCDD exposure. These enzyme activities were directly measured in dorsolateral prostates obtained on PND 21 from male offspring exposed to vehicle or a single, maternal dose of TCDD (0.75 μg/kg) administered on GD 15. No effect of *in utero* and lactational TCDD exposure was found for the type 2 5α-reductase activity in the dorsolateral prostate. However, *in utero* and lactational TCDD exposure increased this activity in the rat ventral prostate.[3] No effect of *in utero* and lactational exposure was found on 3α-hydroxysteroid dehydrogenase activity in PND 21 dorsolateral prostates and this enzyme activity was not reduced by perinatal TCDD exposure in the rat ventral prostate.[3]

Equal amounts of the [^{3}H]-polar metabolites were recovered in culture media of dorsolateral prostates obtained from both treatment groups on PND 11, and little or no androstanedione was found at this time point. In dorsolateral prostates obtained from perinatal TCDD-exposed rats on PND 18, on the other hand, exposure to each radiolabled androgen resulted in the formation of significantly more [^{3}H]-androstanedione and less of the [^{3}H]-polar metabolites than were found in vehicle-exposed offspring. These results suggest that *in utero* and lactational TCDD exposure increases the activity of a dorsolateral prostate 17β-hydroxysteroid dehydrogenase isozyme that interconverts DHT and androstanedione. This is further indicated by the moderate decrease in [^{3}H]-DHT, and corresponding increase in [^{3}H]-androstanedione recovered from TCDD-exposed prostates cultured in the presence of [^{3}H]-DHT compared to similarly cultured vehicle-exposed dorsolateral prostates.

Taken together these results indicate that *in utero* and lactational exposure of rats to TCDD alters several aspects of dorsolateral prostate development. The affected dorsolateral prostates are smaller, androgen responsiveness is reduced during the period from PND 14 to PND 21, and the expression of androgen receptors is also reduced compared to dorsolateral prostates from vehicle-exposed male offspring. While DHT formation and activities of the enzymes 5α-reductase and 3α-hydroxysteroid dehydrogenase were not affected, the conversion of DHT to androstanedione by 17β-hydroxysteroid dehydrogenase was increased by *in utero* and lactational TCDD exposure. This enzyme reaction is reversible, and can therefore act as molecular switch to buffer changes in intracellular DHT concentration. The TCDD-induced increase in this enzyme activity suggests that there are subtle differences in the way that the TCDD-exposed rat dorsolateral prostate maintains its DHT levels compared to that of the vehicle-exposed rat.

Acknowledgment

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References

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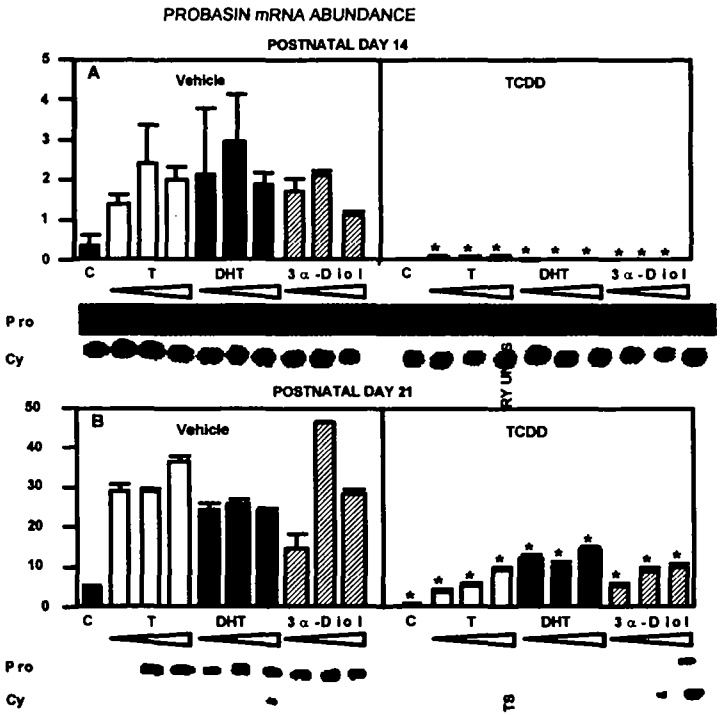

Figure 1. Probasin mRNA expression in dorsolateral prostates obtained from vehicle- and TCDD-exposed rat offspring on PND 11 or 18 and cultured for 24 hours in the absence of androgens, and then with graded concentrations of testosterone, DHT, and 3α-Diol for 48 hours until PND 14 or 21.

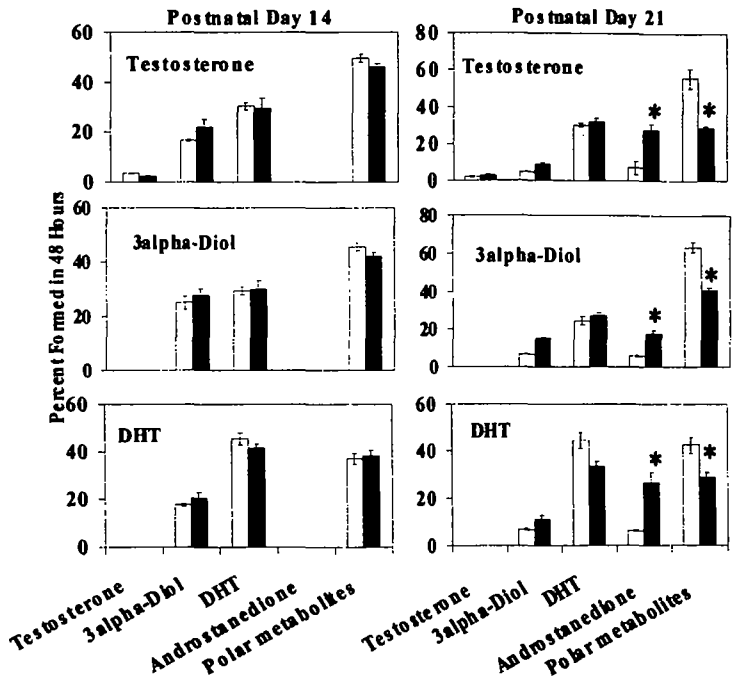

Figure 2. Androgen metabolism in dorsolateral prostates obtained from vehicle- and TCDD-exposed rats on PND 11 or 18 and cultured for 24 hours in the absence of androgen, and then exposed to 10^{-8} M [^{3}H]-testosterone, [^{3}H]-DHT, or [^{3}H]-3α-Diol for 48 hours until PND 14 or 21.