DIOXIN INTERACTS WITH ESTROGEN AND ANDROGEN RESPONSE SYSTEMS TO DISRUPT PROSTATE DEVELOPMENT

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Introduction

Testicular secretion of testosterone begins on gestation day (GD) 15 in rats [1], and is a requirement for differentiation of the prostate, that develop from the cranial region of the embryonic urogenital sinus (UGS). In addition, normal differentiation of the UGS and external genitalia require expression of the enzyme 5α-reductase, that converts testosterone to the more potent dihydrotestosterone (DHT).

Exposure of rat fetuses to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) via a single maternal administration interferes with normal prostate development. However, this effect was not associated with a change in circulating androgen, suggesting a direct effect of TCDD on the prostate rather than an indirect effect via alteration in the secretion of testosterone or conversion of testosterone to DHT [2-4].

The prostate in male rats begins developing on gestation day (GD) 17 in mice and GD 18 in rats. Induction of prostatic epithelial duct formation appears to be regulated by the UGS mesenchyme, which surrounds the UGS epithelium. Epithelial ductal buds develop out the UGS in the dorsocranial, dorsal, lateral and ventral regions. Subsequent epithelial ductal growth and branching continues postnatally, resulting in the formation of the coagulating glands, and the dorsal, lateral and ventral lobes of the prostate [5]

Timms and colleagues have described a computer-based 3-D reconstruction technique for studying fetal development of the prostate [9]. Using this technique, the development of the prostate was found to differ in male Sprague Dawley rat fetuses that occupied different intrauterine positions (IUPs). Male fetuses that occupied an IUP between female fetuses (2F males) had a greater mean area of prostatic buds in the dorsocranial, dorsal and lateral regions relative to male fetuses located between other male fetuses (2M males) [7]. This finding was consistent with prior studies in mice in which as adults, 2F male mice were found to have larger prostates relative to 2M males [10]. The enlarged prostate in 2F males was hypothesized to be mediated by an elevated level of serum estradiol in 2F male fetuses relative to 2M fetuses, due to transport of estradiol from adjacent female fetuses [11,12]. This hypothesis was confirmed in a study in which estradiol was experimentally elevated by 50% in male mouse fetuses (via maternal administration), and the estrogen-treated males showed both a significant increase in the number of prostatic ducts and significantly larger ducts during fetal life, as well as enlarged prostates in adulthood [6].

A number of additional studies have now shown that a small increase in estrogenic activity in male mouse fetuses, due to maternal exposure to very low doses of either estrogenic drugs or environmental estrogens in plastic or pesticides, results in a permanent increase in prostate size and an increase in prostatic androgen receptors during postnatal life [6,13-15]. A primary effect of estrogen in the prostate is thus to increase the sensitivity of cells to androgen.

We have developed a primary cell culture system to study the responses of fetal mouse urogenital sinus mesenchyme cells to hormonal perturbations and xenobiotics.

Methods and Materials

Male mouse fetuses were collected on gestation day 17 and the urogenital sinuses (UGSs) were removed as described [6,7]. The UGS tissue was disrupted by digestion for 30 to 50 min with 3 mg/ml collagenase type I (Sigma) at 37 C in a shaking water bath. Tissues were further disrupted by manual pipetting. To separate mesenchymal cells from clumps of epithelium, the digested tissues were allowed to settle briefly before collection of the suspended mesenchymal cells. Cells were grown in RPMI-1640 (Gibco BRL) supplemented with 2 mM L-glutamine, 100 units penicillin G sodium/ml, 100 mg streptomycin sulfate/ml, 0.25 mg fungizone/ml, and 10% (v/v) fetal bovine serum (FBS) (Harlan).

For hormone treatments, first passage cells were seeded onto 24-well plates in RPMI-1640 supplemented with 2 mM L-glutamine, 100 units penicillin G sodium/ml, 100 mg streptomycin sulfate/ml, 0.25 mg fungizone/ml, 5 % (v/v) charcoal-stripped fetal bovine serum, 5 % (v/v) charcoal-stripped horse serum, insulin transferrin selenium (ITS) supplement (Cambrex), and 200 pg/ml DHT.

Expression of androgen receptor (AR) mRNA was measured by real time RT-PCR as described [8] on an ABI Prism 7700 sequence detection system (PE Applied Biosystems) using the TaqMan EZ RT-PCR kit according to the manufacturer's instructions (PE Applied Biosystems). Amount of androgen receptor mRNA is expressed relative to the total RNA isolated from each sample.

For detection of AR mRNA, we used the forward primer 5'-TGTCAAAAGTGAAATGGGACC-3', the reverse primer 5'-TGGTACTGTCCAAACGCATGT-3', and the probe 5'-TGGATGGAGAACTACTCCGGACCTTATGGG-3'. The amplicon spans bases 1494 to 1587 of mouse AR, which includes the junction of exons 1 and 2.

Results and Discussion

We examined the effects of a wide range of doses of estradiol on AR mRNA levels in primary culture of fetal mouse UGS mesenchyme cells. The cells respond to small increases in estradiol with increases in growth, measured as total RNA (Figure 1A). Estradiol also induces expression of androgen receptor mRNA, in agreement with previous *in vivo* studies (Figure 1B). These results support the hypothesis that the effects of estrogens on prostate development are mediated through direct effects on the mesenchymal cells of the UGS. The xenoestrogen bisphenol A (BPA) has effects similar to estradiol on mesenchyme cell growth and AR mRNA expression (Figure 2).

TCDD has been shown to inhibit estrogen-induced responses in several tissues [16,17]. As described above, 2F male fetuses have higher levels of serum estradiol relative to 2M male fetuses. Timms et al. [18] reported that exposure of pregnant rats to a single dose of TCDD significantly reduced serum estradiol in 2F male fetuses but not in 2M male fetuses. TCDD also significantly interfered with initial budding and subsequent growth of the prostate in 2F males, while there was no effect of TCDD on the prostate in 2M males. Similar to findings from other studies, there was no effect of TCDD on serum testosterone levels. While the ventral lobe is the most commonly studied part of the prostate, in the study by Timms, there were no effects of TCDD in the ventral lobe, while significant effects were found for the dorsocranial, dorsal and lateral lobes. Taken together, these findings suggest that at least some of the effect of TCDD on the developing prostate may be mediated by a decrease in the estrogen-mediated increase in androgen receptor (AR) gene activity and AR protein, since TCDD results in a decrease in serum estradiol, and estradiol increases prostate AR mRNA and protein levels.

There are implications for the findings that very low doses of estrogenic chemicals stimulate prostate growth during fetal life, and that low levels of TCDD in fetuses can interfere with this stimulatory effect of estrogen. TCDD and estrogenic chemicals act as ligands for transcription factors. For chemicals that bind reversibly to cytoplasmic or nuclear receptors, the assumptions underlying current methods of risk assessment are invalid. Two such assumptions are that dose-response curves are monotonic, and there is a threshold dose below which no effects occur [19]. Basing estimates of doses that are safe for human exposure on flawed assumptions has led to dramatic errors in the predicted safe dose for a number of estrogenic chemicals, including the monomer used in the manufacture of polycarbonate plastic, bisphenol A [20]. The stimulating effects of very low doses of bisphenol A, previously assumed to be without effect, on the fetal prostate, and the interference of TCDD with these effects, are currently being examined.

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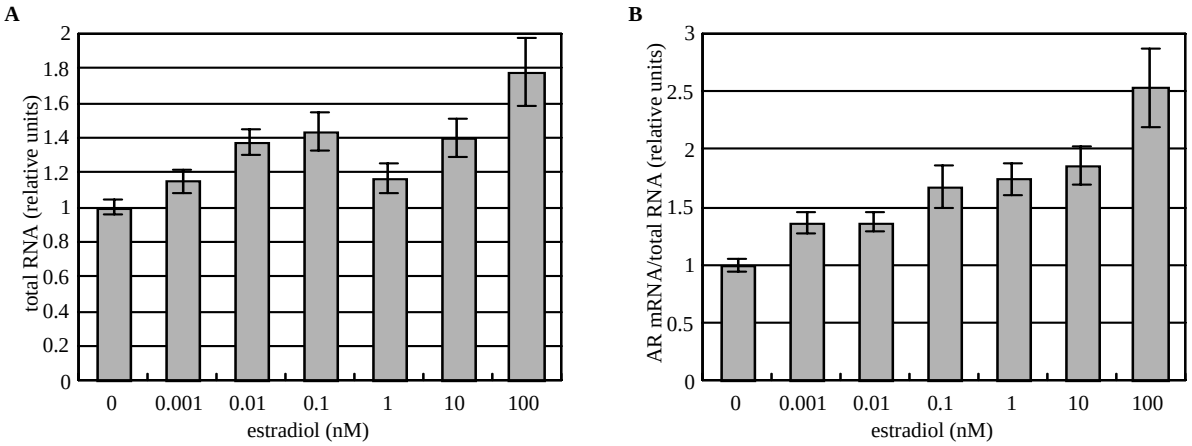

Figure 1. Estradiol induces growth and androgen receptor (AR) mRNA expression in primary cultures of fetal mouse urogenital sinus (UGS) mesenchyme cells. 0.27 pg/mL estradiol is equal to 1 pM. A) Total RNA per well increases with increasing dose of estradiol. Error bars represent one standard error of the mean. B) Androgen receptor mRNA expression relative to total RNA increases with increasing dose of estradiol. Error bars represent one standard error of the mean.

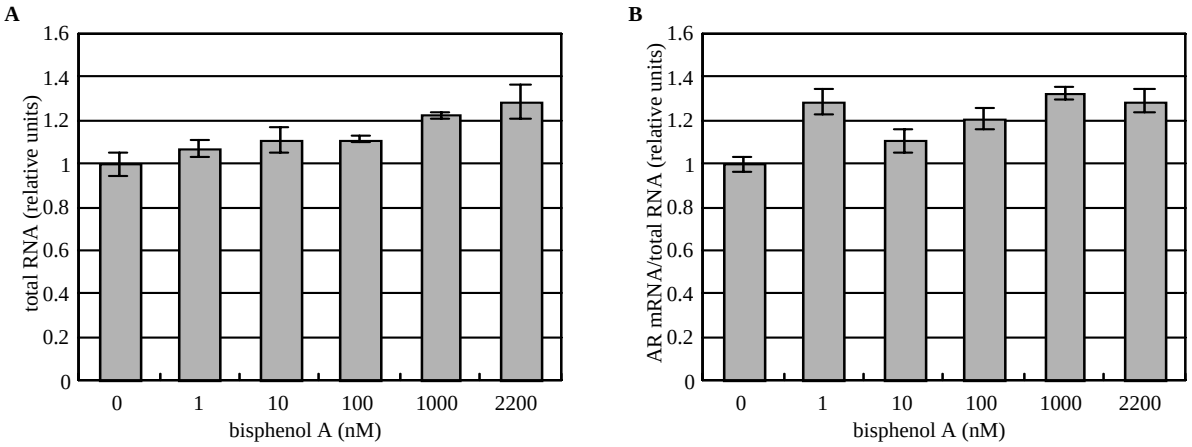

Figure 2. Bisphenol A (BPA) induces growth and androgen receptor (AR) mRNA expression in primary cultures of fetal mouse urogenital sinus (UGS) mesenchyme cells. A) Total RNA per well increases with increasing dose of BPA. Error bars represent one standard error of the mean. B) Androgen receptor mRNA expression relative to total RNA increases with increasing dose of BPA. Error bars represent one standard error of the mean.

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