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In utero exposure to the environmental androgen trenbolone masculinizes female Sprague–Dawley rats

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Abstract

Recently, the occurrence of environmental contaminants with androgenic activity has been described from pulp and paper mill effluents and beef feedlot discharges. A synthetic androgen associated with beef production is trenbolone acetate, which is used to promote growth in cattle. A primary metabolite, 17β Trenbolone (TB), has been characterized as a potent androgen in both in vitro and in vivo studies with rats. The current study was designed to characterize the permanent morphological and functional consequences of prenatal TB exposure on female rats compared with those produced in an earlier study with testosterone propionate (TP). Female rat offspring were exposed to 0 mg/day, 0.1 mg/day, 0.5 mg/day, 1.0 mg/day, or 2.0 mg/day TB on gestational days 14–19. The 0.5 mg/day, 1.0 mg/day, or 2.0 mg/day TB groups displayed increases in neonatal anogenital distance (AGD) which persisted in the high dose group. Puberty was delayed in the high dose group and there were increased incidences of external genital malformations and the presence of male prostatic tissue in the 0.5 mg/day, 1.0 mg/day, or 2.0 mg/day groups. These changes were associated with amniotic fluid concentrations of TB that compare favorably with concentrations known to be active in both in vitro systems and in fish.

Keywords: Trenbolone; EDC; Androgen; Masculinization; Female; Reproductive development

1. Introduction

Since the 1990s, there has been a rising scientific and regulatory interest in environmental chemicals capable of interfering with the endocrine systems of wildlife species and humans (Colborn and Clement, 1992). Although initial research has focused mostly on environmental estrogens, androgenic activity has been described in water from pulp and paper mills and concentrated animal feed operations in the U.S. and Europe (Orlando et al., 2004; Parks et al., 2001; Radl et al., 2005; Durhan et al., 2006; Ellis et al., 2003). Suspected androgenic chemicals associated with pulp and paper mill effluents bound to the androgen receptor (AR) and induced androgen-dependent gene expression in vitro (van den Heuvel et al., 2006; Parks et al., 2001; Larsson and Forlin, 2002). In addition, female mosquito fish (*Gambusia holbrooki*) collected from contaminated rivers were masculinized (Parks et al., 2001).

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Recently, the anabolic steroid trenbolone acetate, used extensively as a growth promoter for farm animals, has been added to an expanding list of environmental androgens (Purdom et al., 1994; Wilson et al., 2002; Jegou et al., 2001; Ankley et al., 2003). The biologically active forms of this androgenic chemical can be comparatively persistent, with a half-life of approximately 260 days (Schiffer et al., 2001). A recent study examining cattle feedlot discharge showed a high level of androgenicity, and wild-caught fathead minnows (*Pimephales promelas*) from a pond adjacent to the site displayed morphological (50% reduction in testis weight) and endocrine (abnormal testosterone/estradiol ratios) alterations as compared to fish from an uncontaminated site (Orlando et al., 2004; Ankley et al., 2003).

One androgenic metabolite of trenbolone acetate, 17β trenbolone (TB) or simply trenbolone, is as potent in vitro and in vivo as are the most potent natural and synthetic androgens (Wilson et al., 2002). Other metabolites include the biologically-active 17α trenbolone and the inactive metabolite, trendione. In vitro experiments, TB binds human and fish androgen receptors with high affinity and induces androgen-dependent gene expression in MDA-KB2 cells at concentrations similar to those for dihydrotestosterone (DHT). In vivo androgenicity testing using the Hershberger (castrate-immature male rat) assay shows that TB is as potent as testosterone propionate (TP) in inducing growth of the androgen-dependent levator ani-bulbocavernosus muscles (LABC) (Wilson et al., 2002). However, in certain tissues, such as the ventral prostate and seminal vesicle, TB (sc) is less effective than equivalent doses of TP in stimulating growth in the immature-castrate male rat. This tissue-specific response is potentially due to the fact that TB, unlike testosterone (T), does not appear to be activated to a more potent androgen by 5α reductase, an enzyme present in high concentrations in tissue such as the ventral prostate and seminal vesicle, but not in the LABC (Wilson et al., 2002; Sundaram et al., 1995; Toth and Zakar, 1986). Hence, TB is described as having anabolic activity (effect on muscles) equivalent to T, but less androgenic (effect on prostate) activity. These chemical properties are not unique to TB and have been seen with many other synthetic C-19 norandrogens.

Prenatal exposure of female rodents to exogenous androgens results in both physiological and behavioral masculinization although the effects vary with the timing of exposure (Wolf et al., 2002; Greene et al., 1939; Huffman and Hendricks, 1981; Slob et al., 1983; Rhees et al., 1997; Hotchkiss et al., 2007; Swanson et al., 1965). Sexual differentiation of the reproductive tract is most sensitive to disruption from days 14–19 in the rat, whereas behavioral sex differentiation is later during perinatal life. The external and internal morphological tissues masculinized by prenatal androgen exposure have been extensively described in rodents. These include the anogenital distance (AGD) and the prepubertal nipple/areolae number. AGD is defined as the distance between the genital papilla and the anus; male rodents have AGDs that are approximately twice those of females (Vandenbergh and Huggett, 1995; Gray et al., 1999). Areolae (areolas) are dark areas surrounding the nipple bud and are indicative of adult nipples. Female rats typically have 12 nipples whereas males have none. Both of these biomarkers are altered with prenatal exposure to androgens or antiandrogens in females and males, respectively (Gray et al., 1999). Internally, normal development of the Wolffian duct derivatives (sex accessory tissues and epididymis) and gubernacular ligaments are influenced by androgens.

In this study, our objective was to characterize the effects of prenatal exposure to trenbolone on female rat reproductive development and measure the serum and amniotic fluid concentrations of the active androgen in order to relate internal exposure levels of TB to the severity of the effects. Effects assessed included changes in biomarkers of prenatal androgen exposure (AGD and areolas), onset of puberty, reproductive potential, and malformations in adult females.

2. Methods

2.1. Animals

Pregnant Sprague–Dawley rats (Charles River Breeding Laboratory, Raleigh, NC) were shipped on the day after mating and housed individually in clear plastic cages (20 cm × 25 cm × 47 cm) with laboratory grade pine shavings as bedding (Northeastern Products, Warrensburg, NY). The day after mating was designated day 1 of gestation. Animals were provided Purina Rat Chow (5008 during pregnancy and lactation and 5001 as juveniles and adults) and filtered (5 microns) water, ad libitum, in a room with a 14:10 h (light/dark, lights off at 11:00 a.m. EST) photoperiod and temperature of 20–22 °C with a relative humidity of 45–55%.

On gestational day 13 (GD 13) animals were weight ranked and assigned randomly to treatment in a manner that provided similar means in body weights for the different treatment groups. Pregnant rats were injected with laboratory-grade corn oil (CAS # 8001-30-7, Sigma, lot # 70K0127) or 17β trenbolone (TB) (CAS # 10161-33-8, Sigma, lot # 60K16611; purity ≥98%) subcutaneously (sc) from GD 14–19 at 0 mg/day, 0.1 mg/day, 0.5 mg/day, 1.0 mg/day, or 2.0 mg/day in 0.1 ml of corn oil. We elected to use this route of exposure and this dosing regime so we could compare the results of this study to those previously reported from this laboratory using TP