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Effects Of *In Utero* and Lactational Exposure to 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) on Serum Androgens and Steroidogenic Enzyme Activities in the Male Rat Reproductive Tract

Gerard M. Cooke,[1]* Christopher A. Price[2] and Richard J. Oko[3]

[1]Toxicology Research Division, Food Directorate, Health Canada, and Reproductive Biology Unit, and Departments of Cellular and Molecular Medicine and Obstetrics and Gynaecology, University of Ottawa, Banting Research Centre, 2202D1 Tunney's Pasture, Ottawa, Ont., K1A 0L2 Canada ; [2]Centre de recherche en reproduction animale (CRRA), Departement d'Anatomie et Physiologie, Faculté de Médecine Vétérinaire, Université de Montréal, CP 5000 St.-Hyacinthe, Qué., J2S 7C6 Canada and [3]Department of Anatomy and Cell Biology, Queen's University, Kingston, Ont., K7L 3N6 Canada

2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) has been shown to impair reproductive function of males in animal models, possibly due to a reduction in serum androgen levels. Thus, TCDD may alter the testosterone biosynthetic pathway in the testis or the conversion of testosterone to 5α-dihydrotestosterone (DHT) in androgen target tissues. Pregnant Sprague Dawley rats were gavaged with TCDD (0, 0.2 or 1.0 μg/kg) on day 15 of gestation only. TCDD caused a reduction in the body weight gain of the dams in both dose groups and a significant reduction in litter size in the higher dose group. Litters delivered normally and TCDD exposed male offspring grew at the same rate as controls. Males were sacrificed at 15, 30, 45, 60, 90 and 120 d of age. Steroidogenic enzyme activities were determined in testicular microsomes and androgen target tissue nuclear fractions. Serum androgens were measured by radioimmunoassay (RIA). At 30 d of age, rats exposed to 1.0 μg/kg TCDD exhibited lower 17-hydroxylase activity ($P < 0.05$) and lower caput-corpus epididymal weights ($P < 0.05$). At 45 d of age, the same treatment resulted in testicular 3β-HSD, 17β-HSD and 5α-reductase activities that were significantly greater ($P < 0.05$) but, conversely, serum androgens were one quarter the values evident in controls ($P < 0.05$). At the other ages, no differences were observed in serum androgens and, with the exception of lower 17β-HSD activity at 90 d of age ($P < 0.05$), no other differences in testicular steroidogenic enzyme activities were found. 5α-reductase activities in the androgen target tissues were also unchanged. Histological examination of testes showed that the spermatogenic profile was identical to controls at all ages.

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INTRODUCTION

In recent years, organochlorine food and environmental contaminants have been implicated in impaired fertility in humans, wildlife species and in laboratory animal models [1–3]. For humans not occupationally exposed, exposure to organochlorines is greatest during fetal and lactational stages of development. After weaning, exposure is predominantly due to contamination of food [4]. The persistence of organochlorine chemicals in the environment and their resistance to metabolism and clearance from the body, leads to an increasing body load throughout life. In the studies described below, we used 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) because this compound has been used as a representative of the dioxin family of compounds due to its toxicity, its

*Correspondence to G. M. Cooke. Tel. 957 0990; Fax: 941 6959; e-mail: gerard_cooke@hc-sc.gc.ca.
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presence in foodstuffs, its occurrence in humans and also because it is used as the standard for toxic equivalency factors (TEFs) [4, 5]. Also, TCDD has been used in many studies of the effects of dioxin exposure on male reproductive function [6–20]. These studies have followed two main designs; treatment of adult male rats with large single doses [6–9] or prolonged and repeated exposure [10, 11] and exposure to short term or single doses during fetal development [12–20]. The latter types of studies have focused on dosing on day 15 of gestation, the time when the fetal rat testis has just been formed. Thus, the developing reproductive tract in the fetus and neonate is especially at risk from organochlorines since it is at these times that the greatest exposure occurs. In rodent models, exposure to TCDD *in utero* and during lactation (IUL) has resulted in impaired fertility, reduced sperm numbers in ejaculates, demasculinized mating behaviour, reduced serum testosterone and LH levels and reduced androgen target tissue weights [12–20]. Many of the alterations in male reproductive function resulting from TCDD IUL could be consequent to the reduced serum testosterone concentration [12–20], especially if testosterone biosynthesis was a target for TCDD action [21]. Therefore, we have examined the effects of TCDD IUL on the steroidogenic enzymes in the male rat reproductive tract that are responsible for the conversion of pregnenolone to testosterone in the testis and also on the activity of 5α-reductase, which was measured in immature testis and in androgen target tissues because 5α-dihydrotestosterone (DHT) is necessary for testicular development and for androgen target tissue function [22–26].

EXPERIMENTAL

Materials

Unlabelled steroids were purchased from Steraloids, Wilton NH and tritiated steroids [1,2,6,7-^{3}H]progesterone (109.5 Ci/mmol), [1,2,6,7-^{3}H]dehydroepiandrosterone DHEA, (89.2 Ci/mmol), [7-^{3}H]4-androstenedione (24.5 Ci/mmol) and [1,2,6,7-^{3}H]testosterone (85.0 Ci/mmol) were bought from Dupont/NEN, Boston MA. Organic solvents were from BDH, Montreal, QC. Nicotinamide cofactors were from Sigma, St. Louis, MO. Plastic coated Whatman™ PE SIL G silica gel chromatography plates were purchased from Chromatographic Specialties, Montreal, QC. TCDD (99.5% pure by gas chromatography) was a gift from Dr. A.E. Pohland, US FDA, Washington DC. All other chemicals were of reagent grade.

Animals

Pregnant Sprague Dawley rats (approximately 300 g) were purchased from Charles River Canada, St. Constant QC, randomly assigned to treatment groups and then gavaged once only with TCDD in corn-oil at 0, 0.2 or 1.0 μg/kg body weight on day 15 of pregnancy ($n = 5$ per treatment group). This treatment regimen leads to exposure of the fetuses *in utero* and during lactation due to transfer of TCDD in breast milk (IUL exposure). Body weight gain of the dams was recorded daily until delivery of the offspring. Male offspring (five per treatment group, one from each litter as far as was possible) were sacrificed at 15, 30, 45, 60, 90 and 120 d of age by exsanguination under Halothane anaesthesia. Blood was obtained via cardiac puncture and serum separated by centrifugation. Body weights, testes weights and androgen target tissue weights were recorded. All tissues were frozen at −20°C until assay for steroidogenic enzyme activities. All animal manipulations were done in accordance with the Guidelines of the Canadian Council on Animal care.

Tissue preparation and enzyme assays

At the time of assay, testes were thawed and homogenized in a motor-driven Potter–Elvejhem homogenizer in Tris–HCl (50 mM) buffer, pH 7.5, containing sucrose (0.25 M), KCl (25 mM), $MgCl_2$ (5 mM), mercaptoethanol (7 mM). The homogenate was centrifuged at $10{,}000 \times g$ for 10 min at 4°C (Beckman L7 Ultracentrifuge, Beckman, Montreal, QC), and the supernatant recentrifuged under the same conditions to yield a post-mitochondrial supernatant. The microsomal fraction was obtained by centrifuging the post-mitochondrial supernatant at $176{,}000 \times g_{max}$ for 1 h at 4°C. Microsomes were resuspended in Tris–HCl buffer, pH 7.5 and used immediately for assessments of steroidogenic enzyme activities. Protein was estimated using the method of Lowry *et al.* [27].

Prostates, seminal vesicles and caput-corpus epididymides were homogenized in phosphate buffer and the nuclear fraction obtained by centrifugation as described previously [28].

Radiometric enzyme assays were done according to the modified methods of Cooke [29]. For 17-hydroxylase activity, aliquots of microsomal fraction were added to Tris–HCl buffer, pH 7.5, (3 ml) containing progesterone (1 μM, 40,000 cpm ^{3}H) (1 cpm = 0.0167 Bq), and NADPH (0.25 mM) at 37°C in a reciprocating water bath. After 30, 60 and 90 min, 1.0 ml was withdrawn and transferred to glass screw-cap test tubes containing hexane (5 ml) and carrier steroids (progesterone, 17-hydroxyprogesterone, 4-androstenedione, and testosterone; 30 μg each). The tubes were vortexed vigorously to terminate the reactions and extract the steroids and then centrifuged to achieve phase separation ($800 \times g$, 10 min). The organic phase was transferred to conical tubes and evaporated (Savant, Speedvac Plus, Fisher Scientific, Ottawa Ont.). The residue was applied to plastic coated thin-layer plates (Whatman PE SIL G)

and developed in chloroform–acetone (9:1 v/v). Carrier steroids were visualized by ultraviolet illumination and exposure to iodine vapor. The regions corresponding to the carrier steroids were excised and quantified by scintillation counting. The combined quantities of radiolabeled 17-hydroxyprogesterone and 4-androstenedione were used to determine 17-hydroxylase activity (testosterone and other steroids were produced in negligible quantities). The activity of C_{17-20} lyase was measured in the same way except that the steroid substrate was 17-hydroxyprogesterone and the quantity of 4-androstenedione produced was used to calculate C_{17-20} lyase activity. 17β-hydroxysteroid dehydrogenase (17β-HSD) activity was measured using 4-androstenedione as the steroid substrate and the quantity of testosterone produced was used to determine activity. For the measurement of 3β-HSD activity, DHEA was the steroid substrate, NAD^+ was the cofactor, the pH of the incubation was 8.4, carrier steroids were; DHEA, 5-androstenediol, 4-androstenedione and testosterone, and the only product was 4-androstenedione.

Testosterone metabolism by immature testis microsomes and nuclear fraction from prostates, epididymides and seminal vesicles from all age groups was measured exactly as previously described [28]. 5α-reductase activity was the combined amount of DHT and 5α-androstanediols produced.

Preliminary studies were done to optimize conditions for all enzymes with respect to substrate and protein concentrations and linearity of reaction with time. Reaction rates were obtained by linear regression analysis (r>0.95).

Androgen radioimmunoassays

Serum androgens (testosterone plus DHT) were measured by a direct RIA employing a previously validated antibody against testosterone [30] and which cross-reacts strongly with DHT (77%). Steroid was displaced from serum binding proteins by incubating 30 μl sample (made up to 100 μl with 0.05 M phosphate buffer containing 0.9% NaCl and 0.1% gelatin, pH 7.2) with estradiol (400 ng in 100 μl buffer) for 30 min at room temperature, followed by addition of antibody and tracer (100 μl each). The following day, antibody-bound tracer was counted after standard charcoal-dextran separation. Dilutions of sera from rats (2–50 μl) were parallel to the standard curve. Androgen values in rat serum determined with the direct assay were significantly correlated with ($r = 0.95$, $P < 0.01$, $n = 23$) and not significantly different from values obtained after diethyl ether extraction. All samples were run in one assay in which the limit of quantization was 33 pg/ml and the intra-assay CV was 8%.

To determine testosterone and DHT concentrations separately, 100 μl serum were extracted twice with 1 ml petroleum ether:ethyl acetate (11:2), and the residue dissolved in ethylene glycol-saturated iso-octane. The extracts were then applied to a glass column containing celite equilibrated as described [31]. The columns were washed with 3.5 ml iso-octane, then 3.5 ml 5% ethyl acetate in iso-octane (DHT fraction), followed by 3.5 ml 15% ethyl acetate in iso-octane (testosterone fraction). All serum samples were incubated with tritiated DHT and tritiated testosterone for 30 min at room temperature prior to extraction, to allow estimation of extraction efficiency. Control serum samples received either DHT or testosterone recovery tracer, and were extracted as described; 5% of the DHT trace was eluted in the testosterone fraction and less than 5% of the testosterone trace was eluted in the DHT fraction. Mean recovery of testosterone from serum was 68%, and that of DHT was 48%. The appropriate column eluates were dried down, reconstituted in assay buffer, and assayed with the antibody described above against the appropriate standard curve (DHT or testosterone). All samples were run in a single assay for each androgen. The limits of quantization of the assays were equivalent to 60 pg testosterone/ml serum and 30 pg DHT/ml. The intra-assay CV was less than 10% for both assays.

Testis histology

Testes were fixed in formalin and embedded in paraffin. Sections (4 μm) were stained with haematoxylin and eosin.

Statistics

The effects of TCDD on steroidogenic enzyme activities were compared by one-way ANOVA followed by Dunn's multiple comparison test (SigmaStat Version 1.0 for Windows, Jandel Scientific 1992–1994, San Rafael, CA).

RESULTS

Effect of TCDD on pregnancy outcome and development of male offspring

The rate of weight gain in the pregnant control rats was significantly greater compared to growth rates of the TCDD treated dams ($P < 0.05$; Table 1) but all pregnancies delivered on the expected d. Litter sizes in the 1.0 μg TCDD/kg dose group were significantly lower compared to control ($P < 0.05$) and this may have accounted for the reduced rate of weight gain observed in the dams. All the pups appeared normal and no gross malformations or genital abnormalities were observed.

Body and tissue weights of the male offspring at the time of sacrifice are presented in Table 2. TCDD exposure had no effect on the growth rate, testis weights or prostate weights at any time during development. Seminal vesicle weights were higher in

Table 1. *Rate of weight gain, length of gestation and litter sizes for control and TCDD treated pregnant rats*

	Control 0.2 μg/kg	TCDD 1.0 μg/kg	TCDD
Dam weight gain (days 15–21) (g/d, mean ± SE)	17.0 ± 0.9^{a}	13.7 ± 0.7^{b}	13.3 ± 0.8^{b}
Gestation period (d, mean ± SE)	21.4 ± 0.2	21.4 ± 0.2	21.4 ± 0.2
Pups per litter (*n*, mean ± SE)	13.8 ± 0.7^{a}	$11.0 \pm 1.1^{a,b}$	10.4 ± 0.9^{b}

15 dams ($n = 5$ per group) were gavaged on day 15 of gestation only. Body weight was recorded daily from day 15 until the day of delivery. Statistical significance (ANOVA) within rows denoted by different superscripts ($P < 0.05$).

Table 2. *Body and reproductive organ weights of male offspring (n = 5 per group) from pregnant rats gavaged with TCDD on day 15 of gestation*

	TCDD (μg/kg)	Day 15	Day 30	Day 45	Day 60	Day 90	Day 120
Body wt (g, mean ± SE)	Control	28.4 ± 0.6	84.8 ± 1.2	258.8 ± 3.2	391.0 ± 10.7	532.8 ± 13.9	593.2 ± 23.0
	0.2	32.0 ± 1.5	95.0 ± 2.4	260.2 ± 12.8	392.6 ± 11.6	519.2 ± 8.0	626.0 ± 34.6
	1.0	32.4 ± 1.0	96.8 ± 0.8	278.4 ± 5.9	391.8 ± 14.8	536.4 ± 14.0	610.5 ± 14.5
Testis wt. (mg, mean ± SE)	Control	17.0 ± 2.7	218.8 ± 4.1	900.5 ± 65.8	790.0 ± 59.6	991.6 ± 32.0	1033.0 ± 48.0
	0.2	20.3 ± 1.8	247.9 ± 8.9	882.8 ± 53.0	827.0 ± 58.3	991.6 ± 92.6	1034.0 ± 42.0
	1.0	22.9 ± 3.5	241.6 ± 14.9	987.3 ± 41.0	888.0 ± 45.1	889.8 ± 80.2	1000.0 ± 54.0
Prostate wt (mg, mean ± SE)	Control	9.5 ± 1.3	36.3 ± 5.8	169.1 ± 22.5	268.0 ± 42.7	367.0 ± 51.3	391.2 ± 60.0
	0.2	10.3 ± 1.4	35.5 ± 5.1	166.2 ± 18.7	258.6 ± 43.3	418.0 ± 30.7	460.6 ± 54.0
	1.0	6.7 ± 2.1	36.5 ± 5.0	141.8 ± 11.7	207.6 ± 25.8	340.0 ± 37.0	399.2 ± 52.3
Sem. ves. wt (mg, mean ± SE)	Control	4.6 ± 1.3^{a}	53.7 ± 7.5	619.3 ± 63.9	330.6 ± 35.0	303.2 ± 26.0	980.6 ± 63.0
	0.2	14.7 ± 1.4^{b}	47.7 ± 5.7	584.1 ± 66.9	338.0 ± 33.0	289.2 ± 22.0	1090.0 ± 95.0
	1.0	17.8 ± 2.2^{b}	42.7 ± 1.7	543.3 ± 48.3	359.4 ± 20.4	337.5 ± 9.2	1159.0 ± 56.0
Caput-corpus epid. wt (mg, mean ± SE)	Control	35.8 ± 2.9	100.0 ± 3.4^{a}	347.5 ± 33.8	459.2 ± 48.6	588.5 ± 35.6	883.0 ± 28.0
	0.2	39.6 ± 1.9	84.2 ± 6.7^{a}	396.7 ± 22.6	516.0 ± 45.6	571.0 ± 76.5	927.0 ± 45.0
	1.0	40.5 ± 2.3	70.5 ± 5.3^{b}	379.2 ± 37.7	488.8 ± 9.9	642.2 ± 37.3	844.0 ± 56.0

Statistical significance (ANOVA) within columns denoted by different superscripts ($P < 0.05$).

TCDD exposed males on day 15 ($P < 0.05$) and epididymal weights were lower in TCDD exposed males on day 30 ($P < 0.05$). At all other time points, no significant differences were observed. The apparently greater seminal vesicle weights observed for day 45 were due to an error where weights were recorded without removal of the seminal fluid.

Effect of TCDD IUL on testicular and sex accessory organ steroidogenic enzymes

The developmental profile of 17-hydroxylase specific activity in testicular microsomes from control rats demonstrated higher activity in younger animals compared with adult ages (Fig. 1). Similar profiles were evident for the rats exposed to TCDD IUL and although the activity was generally lower than in controls, it was only at 30 d of age and in the 1.0 μg/kg dose of TCDD, that the 17-hydroxylase activity was significantly lower (70% of the control value; $P < 0.05$). At all other time points, 17-hydroxylase activity was not different compared with controls. C_{17-20} lyase activity was highest at 45 and 60 d of age in control rats but exposure to TCDD IUL had no effect on the activity (Fig. 2).

In control rats, the level of 3β-HSD activity was highest at 45 d of age and at this age, exposure to 1.0 μg/kg TCDD IUL resulted in a two-fold increase in 3β-HSD activity ($P < 0.05$; Fig. 3). Similarly, at 45 d of age, 17β-HSD activity was also two fold higher in this TCDD exposed group compared with control and, at 90 d of age, 17β-HSD activity was sig-

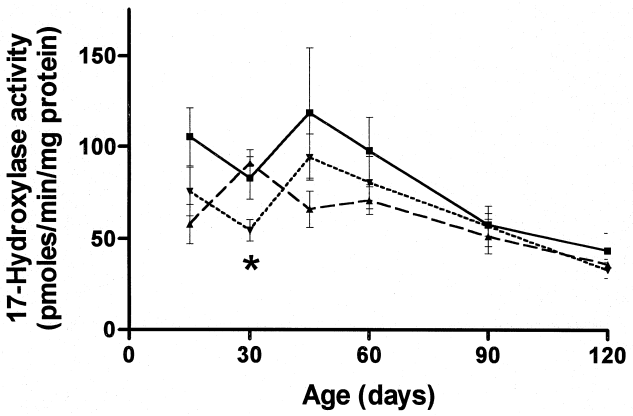

Fig. 1. 17-Hydroxylase activity in testis microsomes from male offspring of dams gavaged with TCDD in corn-oil at 0.0 (solid line), 0.2 (dashed line) or 1.0 (dotted line) μg/kg body weight on day 15 of gestation. Males ($n = 5$ per treatment group) were sacrificed at 15, 30, 45, 60, 90 and 120 d of age. Testis microsomes were incubated with progesterone (1 μM, containing 40,000 cpm ^{3}H-progesterone) and NADPH (0.25 mM) for 0, 30, 60 and 90 min as described in Section 2. Specific enzyme activity (pmoles 17-hydroxyprogesterone plus 4-androstenedione produced/min/mg microsomal protein) was determined by linear regression analysis. Values are means ± SE. *Control and 0.2 μgTCDD/kg activities are significantly greater than 1.0 μgTCDD/kg activity ($P < 0.05$).

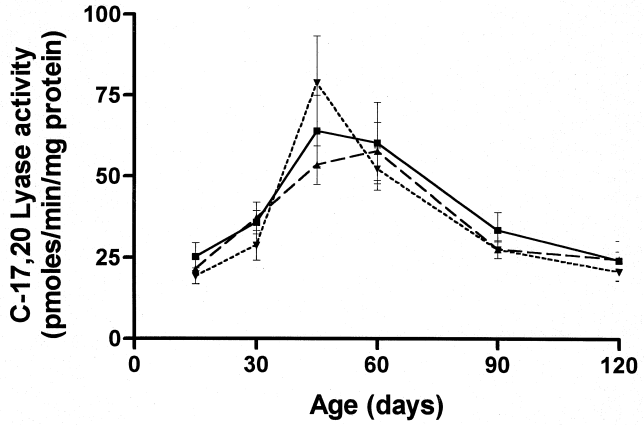

Fig. 2. $C_{17,20}$ Lyase activity in testis microsomes from male offspring of dams gavaged with TCDD in corn-oil at 0.0 (solid line), 0.2 (dashed line) or 1.0 (dotted line) μg/kg body weight on day 15 of gestation. Males ($n = 5$ per treatment group) were sacrificed at 15, 30, 45, 60, 90 and 120 d of age. Testis microsomes were incubated with 17-hydroxyprogesterone (1 μM, containing 40,000 cpm ^{3}H17-hydroxyprogesterone) and NADPH (0.25 mM) for 0, 30, 60 and 90 min as described in Section 2. Specific enzyme activity (pmoles 4-androstenedione produced/min/mg microsomal protein) was determined by linear regression analysis. Values are means ± SE.

nificantly lower compared to control (50% of the control value; $P < 0.05$; Fig. 4).

5α-reductase activity was detectable in the immature rat testis [32–34], and exposure to 1.0 μg/kg TCDD IUL, resulted in threefold higher 5α-reductase activity at 45 d of age compared with control ($P < 0.05$; Fig. 5). In the epididymis, seminal vesicle and prostate, TCDD exposure had no effect on 5α-reductase activity ($P > 0.05$; Fig. 6).

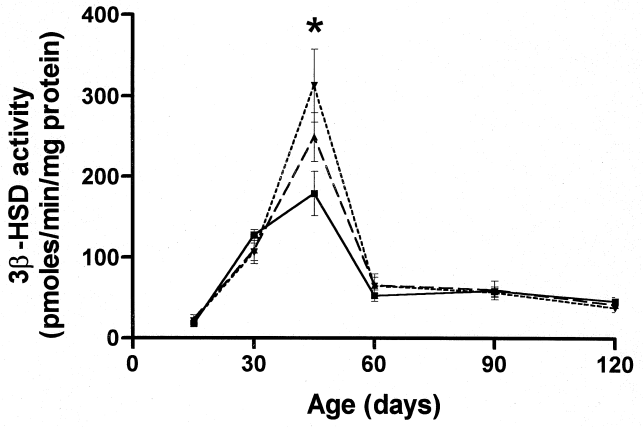

Fig. 3. 3β-HSD activity in testis microsomes from male offspring of dams gavaged with TCDD in corn-oil at 0.0 (solid line), 0.2 (dashed line) or 1.0 (dotted line) μg/kg body weight on day 15 of gestation. Males ($n = 5$ per treatment group) were sacrificed at 15, 30, 45, 60, 90 and 120 d of age. Testis microsomes were incubated with DHEA (1 μM, containing 40,000 cpm ^{3}H-DHEA) and NAD^+ (0.25 mM) for 0, 30, 60 and 90 min as described in Section 2. Specific enzyme activity (pmoles 4-androstenedione produced/min/mg microsomal protein) was determined by linear regression analysis. Values are means ± SE. *Control and 0.2 μgTCDD/kg activities are significantly greater than 1.0 μgTCDD/kg activity ($P < 0.05$).

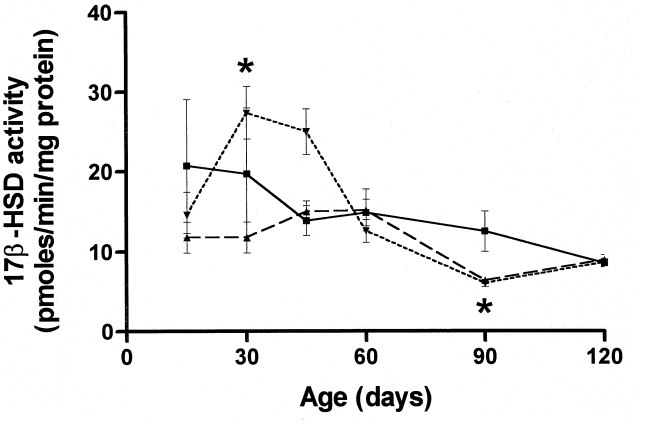

Fig. 4. 17β-HSD activity in testis microsomes from male offspring of dams gavaged with TCDD in corn-oil at 0.0 (solid line), 0.2 (dashed line) or 1.0 (dotted line) μg/kg body weight on day 15 of gestation. Males ($n = 5$ per treatment group) were sacrificed at 15, 30, 45, 60, 90 and 120 d of age. Testis microsomes were incubated with 4-androstenedione (1 μM, containing 40,000 cpm ^{3}H-4-androstenedione) and NADPH (0.25 mM) for 0, 30, 60 and 90 min as described in Section 2. Specific enzyme activity (pmoles testosterone produced/min/mg microsomal protein) was determined by linear regression analysis. Values are means ± SE. *At 45 d of age, control and 0.2 μgTCDD/kg activities are significantly lower than 1.0 μgTCDD/kg activity and at 90 d of age, control activity is significantly greater than both TCDD exposed groups ($P < 0.05$).

Effect of TCDD IUL on serum androgen levels

Serum androgens in the blood samples taken from controls at the time of sacrifice revealed that androgen levels rose sharply beginning at about 30 d of

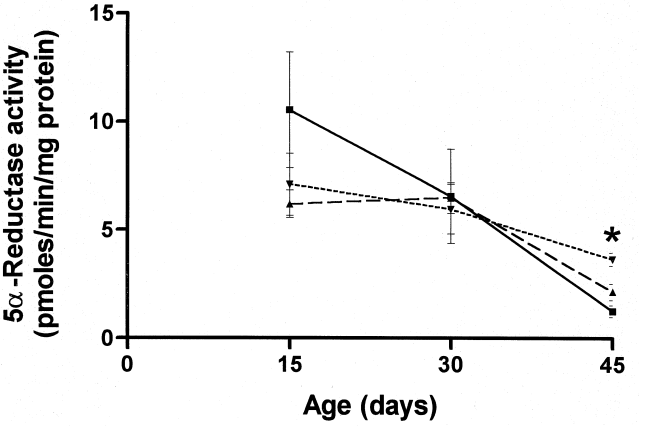

Fig. 5. 5α-Reductase activity in testis microsomes from male offspring of dams gavaged with TCDD in corn-oil at 0.0 (solid line), 0.2 (dashed line) or 1.0 (dotted line) μg/kg body weight on day 15 of gestation. Males ($n = 5$ per treatment group) were sacrificed at 15, 30 and 45 d of age. Testis microsomes were incubated with testosterone (0.1 μM, containing 40,000 cpm ^{3}H-testosterone) and NADPH (0.25 mM) for 0, 30, 60 and 90 min as described in Section 2. Specific enzyme activity (pmoles dihydrotestosterone plus 3α/β-diols produced/min/mg microsomal protein) was determined by linear regression analysis. Values are means ± SE. *At 45 d of age, control and 0.2 μgTCDD/kg activities are significantly lower than 1.0 μgTCDD/kg activity ($P < 0.05$).

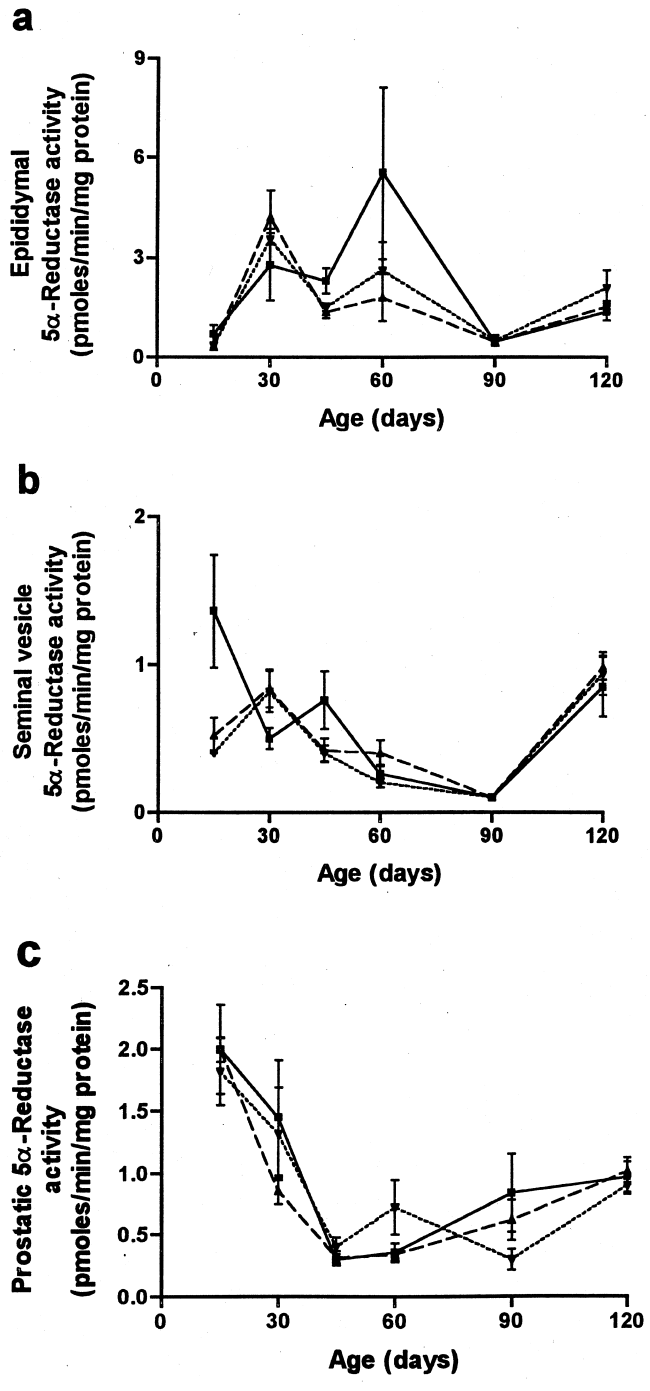

Fig. 6. 5α-Reductase activity in nuclear fractions from caput-corpus epididymides, seminal vesicles and prostates from male offspring of dams gavaged with TCDD in corn-oil at 0.0 (solid line), 0.2 (dashed line) or 1.0 (dotted line) μg/kg body weight on day 15 of gestation. Males ($n = 5$ per treatment group) were sacrificed at 15, 30, 45, 60, 90 and 120 d of age. Nuclear fractions were incubated with testosterone (0.1 μM, containing 40,000 cpm ^{3}H-testosterone) and NADPH (0.25 mM) for 0, 30, 60 and 90 min as described in Section 2 and [28]. Specific enzyme activity (pmoles dihydrotestosterone plus 3α/β-diols produced/min/mg nuclear fraction protein) was determined by linear regression analysis. Values are means ± SE.

age, as has been shown by others [35–37]. Exposure to 1.0 μg/kg TCDD IUL resulted in significantly lower serum androgen levels at 45 d of age and significantly higher levels at 120 d of age ($P < 0.05$). At all other time points, serum androgens in TCDD exposed rats were not significantly different compared with controls (Fig. 7). In view of the higher 5α-reductase activity in the testes of 45 d old TCDD treated rats (Fig. 5), serum testosterone and DHT were separated on celite columns and measured by RIA. Mean testosterone levels were significantly lower in rats exposed to 1.0 μg/kg TCDD compared with controls (576 ± 81 vs 2256 ± 550 pg/ml; $P < 0.05$) and DHT levels reflected the same decrease (42.2 ± 7.8 vs 182.4 ± 50.5 pg/ml; $P < 0.05$).

Effect of TCDD IUL on testis morphology

Histological examination of testis morphology revealed no obvious differences between TCDD and control rats at any time point during development. Even at the highest dose of TCDD there appeared to be no increase of apoptotic germ cells in androgen sensitive stages of the cycle of the seminiferous epithelium (stages VII–VIII and XIV). In these stages, an increase in degenerating spermatocytes and spermatids is expected as the earliest morphological indicator of androgen deficiency and is easily detected [38, 39]. Furthermore, at each time point in testicular development (i.e., day 15, 30, and 45 postpartum) the most mature germ cell front seen (e.g., early round spermatids at day 30) and their cellular associations did not change between TCDD exposed

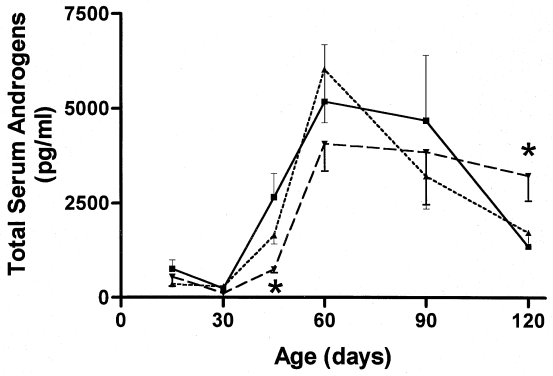

Fig. 7. Total serum androgens in male offspring of dams gavaged with TCDD in corn-oil at 0.0 (solid line), 0.2 (dashed line) or 1.0 (dotted line) μg/kg body weight on day 15 of gestation. Males ($n = 5$ per treatment group) were sacrificed at 15, 30, 45, 60, 90 and 120 d of age and blood was obtained by cardiac puncture under halothane anaesthesia. Total androgens in the serum were measured by radioimmunoassay. Values are means ± SE. *At 45 d of age, the serum androgen levels in the 1.0 μgTCDD/kg group were significantly lower compared with both the controls and 0.2 μgTCDD/kg group ($P < 0.05$) and, at 120 d of age, the serum androgen levels in the 1.0 μgTCDD/kg group were significantly higher compared with both the controls and 0.2 μgTCDD/kg group ($P < 0.05$).

and control rats, indicating that treatment had no effect on delaying the onset of spermatogenesis.

DISCUSSION

The studies presented here have shown that exposure of male fetuses to TCDD on day 15 of pregnancy, resulted in alterations of testicular steroidogenic enzyme activities and serum androgen levels during post natal development and furthermore, that the effects of TCDD are confined to narrow windows of time. At 30 d of age, rats exposed to 1.0 μg/kg TCDD exhibited lower 17-hydroxylase activity and lower caput-corpus epididymal weights but serum androgen levels were normal. At 45 d of age, the same treatment resulted in testicular 3β-HSD, 17β-HSD and 5α-reductase activities that were significantly greater than the levels found in controls but, conversely, serum testosterone and DHT were one quarter the values evident in controls. At the other ages investigated, no differences were observed in serum androgens and, with the exception of lower 17β-HSD activity at 90 d of age, no other differences in steroidogenic enzyme activities were found. The treatment caused no problems with the pregnancy or time of delivery. Post-natally, exposed males grew at the same rate as controls, testicular and prostate weights were normal and the spermatogenic profile was identical to controls at all ages. Consequently, the effect of TCDD on steroidogenic enzymes could not be explained by any delay in development that would have influenced the determination of enzyme activity. It was also apparent, that the effect of TCDD on steroidogenic enzymes was unlikely to be responsible for the decreased serum androgen levels observed here and in other studies [12–20] since, when 17-hydroxylase was decreased by the treatment, androgen levels were normal, and at the time when androgen levels were lower, TCDD had caused increases in 3β-HSD and 17β-HSD and had no effect on the other enzymes. Furthermore, at 45 d of age, when 5α-reductase activity was higher compared with controls, serum DHT levels were lower indicating that the decrease in serum testosterone was not due to enhanced metabolism to 5α-reduced products.

TCDD has been shown to decrease the cytochrome P450 and heme content of the rat testis [40]; inhibit the mobilization of cholesterol to the mitochondria [41] and to reduce Leydig cell number, Leydig cell volume and the quantity of smooth endoplasmic reticulum in Leydig cells [7] and, furthermore, TCDD causes the depression of testicular 17-hydroxylase activity [9]. However, in these studies, the doses of TCDD were higher than used in our studies and were given directly to the male rats. In experiments of similar design to the one used here, TCDD has been shown to delay the onset of puberty, decrease penis size, prostate weight, seminal vesicle weight, cauda epididymal sperm number and ejaculated sperm number [12–20] and sexual behaviour is less masculine and more feminine [14, 16]. Since the changes in these parameters would be attributable to reduced testosterone production, an examination of the testosterone producing enzymes was required. However, the changes in steroidogenic enzyme activities resulting from exposure to TCDD were insufficient to be deleterious to normal testosterone biosynthesis. It has been reported that TCDD causes decreases in LH secretion in male rats [18] that IL-1β levels in the liver are altered by TCDD [42] and that TNFα secretion from peritoneal macrophages is enhanced by TCDD [43]. It is therefore possible that, in the testis, the effects of TCDD are attributable less to the testosterone synthetic capacity and more to the effects of the levels of cytokines and growth factors which would be involved in the formation of the fetal testis and its subsequent function. Recently, a possible explanation for reduced sperm numbers in ejaculates and in the cauda epididymis has been proposed in which TCDD primarily affects epididymal function [20]. In this regard, our data show that epididymal 5α-reductase activity is not affected and therefore, other mechanisms must be involved in the reduction of sperm numbers within the epididymis.

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