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CHANGES IN STEROID HORMONE METABOLISM IN RAT LIVER MICROSOMES FOLLOWING ADMINISTRATION OF 2,3,7,8-TETRACHLORODIBENZO-*p*-DIOXINE (TCDD)

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Abstract—Treatment of male and female rats with 4 × 20 μg/kg of 2,3,7,8-tetrachlorodibenzo-*p*-dioxine (TCDD) led to 2–3-fold increases in liver microsomal cytochrome P450 levels concomitantly with changes in liver microsomal metabolism of 4-androstene-3,17-dione, 5α-androstane-3α,17β-diol and 4-pregnene-3,20-dione. The changes were most pronounced in female rats where some hydroxylase activities increased 3–5-fold. It is suggested that the observed effects following TCDD administration may be related to the endocrine symptoms sometimes seen in patients exposed to this drug.

2,3,7,8-Tetrachlorodibenzo-*p*-dioxine (TCDD) is known to produce fetal malformations, hepatic during the commerical synthesis of the herbicide 2,4,5-trichlorophenoxy acetic acid [1]. TCDD is known to produce fetal malformations, hepatic necrosis, edema, thymic atrophy and hyperkeratosis in laboratory animals [2]. The chloracne and porphyria cutanea tarda reported in a 2,4,5-trichlorophenoxy acetic acid factory have been attributed to contamination of TCDD [3]. In the liver, TCDD was shown to induce δ-aminolevulinic acid synthetase in the chick embryo [4] and increases in cytochrome P450-dependent enzymes in rodents and rabbits [5, 6].

Evidence is accumulating that at least part of the toxic reactions caused by TCDD could be mediated via effects on endocrine systems [7]. Since steroid hormones also serve as substrates for the liver microsomal cytochrome P450 system it was considered of interest to investigate the effects of TCDD on liver metabolism of some common steroid substrates.

MATERIALS AND METHODS

Steroids. 4-[4-^{14}C]androstene-3,17-dione (specific radioactivity, 60 mCi/mmol) was purchased from New England Nuclear (Boston, Mass., USA) and was purified by thin-layer chromatography prior to use. 5α-[4-^{14}C]Androstane-3α,17β-diol was prepared from 4-[4-^{14}C]androstene-3,17-dione as described previously [8]. 4-[4-^{14}C]Pregnene-3,20-dione (specific radioactivity 60 mCi/mmol) was obtained from the Radiochemical Centre (Amersham, England) and was also purified prior to use.

Unlabelled androstenedione, androstanediol and progesterone were kindly given by Dr. J. Babcock, The Upjohn Co., Kalamazoo, Mich., USA. TCDD was a generous gift from Dr. A. Poland, USA.

Treatment of animals. Male and female eight-week-old rats of the Sprague–Dawley strain were kept in a temperature- and light-controlled room (23°, lights on 0600–2000 hr) with free access to food and water. Four rats of each sex were injected intraperitoneally with 20 μg/kg of TCDD in 40 μl of dioxane each day for four days. Control rats of each sex received vehicle only. The rats were killed by decapitation on the fifth day and their livers processed as described below.

Preparation of and incubations with liver microsomes. Following decapitation of the rats, their livers were quickly excised, put into ice-cold Bucher medium, pH 7.4 [9] and weighed. All subsequent work was carried out in a cold-room at 4° and rubber gloves were worn throughout the whole experiment. The livers were homogenized with a Potter–Elvehjem homogenizer equipped with a loosely fitting pestle. The homogenate, 20% (w/v), was centrifuged at 20,000 *g* for 30 min at 4°. The microsomal fraction was prepared by centrifuging the 20,000 *g* supernatant fluid in polycarbonate tubes, at 105,000 *g*, in an L3-50 Beckman ultracentrifuge for 70 min at 4°. The supernatant was decanted into a special container for subsequent destruction. The sediment (microsomal fraction) was suspended in 8 ml of Bucher medium and homogenized with a loosely fitting pestle. The protein concentration of the microsomal fraction was determined according to Lowry *et al.* [10]. Microsomal suspension equivalent to 0.10 g of liver tissue was incubated in 3 ml of Bucher medium with 500 μg of 4[4-^{14}C]androstene-3,17-dione or 4-[4-^{14}C] pregnene-3,20-dione at 37° for 10 min in the presence of an NADPH-regenerating system [8]. Incubations with 5α-[4-^{14}C]androstane-3α,17β-diol were carried out using 200 μg of substrate and microsomal suspension equivalent to 0.50 g of liver tissue in 4 ml of Bucher medium containing an NADPH-regenerating system. The incubation conditions used ascertained conversion of substrate linear with time and enzyme concentration as well as saturation of enzyme with substrate.

Analysis of incubation extracts. The incubation mixtures were extracted and analyzed by thin-layer chromatography and autoradiography as described previously [11, 12]. The solvent systems used allowed separation of all steroid metabolites analyzed. The radioactive zones were localized, scraped off separately

Table 1. Metabolism of 4-[4-^{14}C]androstene-3,17-dione, 6α-[4-^{14}C]androstane-3α,17β-diol and 4-[4-^{14}C]pregnene-3,20-dione and concentration of cytochrome P450 in the microsomal fraction of liver from male and female rats treated with TCDD or vehicle only (Experiment I)

	♂ control	♂TCDD	♀ control	♀ TCDD
4-[4-^{14}C]Androstene-3,17-dione				
7α-hydroxylase	0.72 ± 0.34	1.8 ± 0.37†	0.65 ± 0.09	3.6 ± 0.55‡
6β-hydroxylase	2.2 ± 0.82	3.7 ± 1.5	0.49 ± 0.08	2.1 ± 0.41‡
16α-hydroxylase	1.4 ± 0.57	0.56 ± 0.07*	0	0
17-oxidoreductase	1.1 ± 0.28	1.1 ± 0.25	0.45 ± 0.04	1.0 ± 0.27*
5α-reductase	2.5 ± 1.2	1.7 ± 0.57	18 ± 1.9	11 ± 1.2‡
5α-[4-^{14}C]Androstane-3α,17β-diol				
7α-hydroxylase	0.31 ± 0.04	0.51 ± 0.11*	0.23 ± 0.02	0.79 ± 0.13‡
2β-hydroxylase	0.25 ± 0.02	0.12 ± 0.03‡	0	0.10 ± 0.01
2α-hydroxylase	0.67 ± 0.12	0.18 ± 0.06‡	0.10 ± 0.01	0.44 ± 0.07‡
18-hydroxylase	0.30 ± 0.04	0.32 ± 0.11	0	0.17 ± 0.05
4-[4-^{14}C]Pregnene-3,10-dione				
16α-hydroxylase	1.9 ± 0.29	2.5 ± 0.29*	0.83 ± 0.12	3.10 ± 0.60‡
6β-hydroxylase	0.79 ± 0.20	0.61 ± 0.09	0.29 ± 0.04	0.79 ± 0.24*
5α-reductase	4.8 ± 0.56	2.2 ± 0.51‡	21 ± 0.34	13 ± 2.3†
Cytochrome P450	0.58 ± 0.09	1.38 ± 0.14‡	0.55 ± 0.11	1.06 ± 0.19†

The enzyme activites are expressed in nmol · mg protein^{-1} · min^{-1} and the concentration of cytochrome P450 as nmol · mg protein^{-1} (means ± S.D.; $n = 4$). * = $P < 0.05$, † = $P < 0.01$ and ‡ = $P < 0.001$ when compared to appropriate control.

and measured for radioactivity in an Intertechnique SL-30 liquid scintillation spectrometer. The recovery of radioactivity in the different incubations when comparing the sum of metabolites and residual substrate to the original amount of substrate was always above 95 per cent. The steroid metabolites in the various zones were identified by gas chromatography–mass spectrometry (LKB 2091 instrument) as described in previous publications [11, 12]. Statistical analysis was performed by means of Student's '*t*' test and the significance level was set at 0.05.

RESULTS AND DISCUSSION

Measurements of the amount of metabolites formed from 4-androstene-3,17-dione, 5α-androstane-3α, 17β-diol and 4-pregnene-3,20-dione allowed the calculation of the following enzyme activities: 5α-reductase, 17-oxidoreductase, 6β-, 7α- and 16α-hydroxylases (active on 4-androstene-3,17-dione), 2α-, 2β-, 7α-, 7β- and 18-hydroxylases (active on 5α-androstane-3α,17β-diol) and 5α-reductase, 6β- and 16α-hydroxylases (active on 4-pregnene-3,20-dione).

The metabolism of steroid hormones in the liver microsomal fraction was dramatically changed following treatment with TCDD. The largest changes were observed in female rats where 3–5-fold increases were noted in the following enzyme activities: 7α- and 6β-hydroxylases active on 4-androstene-3,17-dione; 7α- and 2α-hydroxylases active on 5α-androstane-3α,17β-diol; and 16α- and 6β-hydroxylases active on 4-pregnene-3,20-dione. Furthermore, 2β- and 18-hydroxylation of 5α-androstane-3α,17β-diol which were not observed in control females were induced following treatment with TCDD (Table 1). 7α-Hydroxylation of 4-androstene-3,17-dione and 5α-androstane-3α,17β-diol as well as 16α-hydroxylation of 4-pregnene-3,20-dione were also increased in male rats following treatment with TCDD, although not to the same extent as in female rats. In contrast to the situation in female rats, several hydroxylase activities dropped as a consequence of TCDD administration, i.e. 16α-hydroxylation of 4-androstene-3,17-dione and 2β- and 2α-hydroxylation of 5α-androstane-3α,17β-diol (Table 1). Decreases in 5α-reduction of 4-androstene-3,17-dione and 4-pregnene-3,20-dione were noted in both male and female rats. Total liver microsomal cytochrome P450 concentration increased about twice in both sexes following treatment with TCDD.

In conclusion, the present investigation has demonstrated significant changes in cytochrome P450-dependent steroid metabolism in rat liver following administration of TCDD. The observed effects were especially pronounced in female rats. The induced changes in steroid metabolism may offer a possible explanation for the signs of endocrine disturbances (hairfall or hirsutism, diminished libido or potency; cf. [7]) noted in patients with previous exposure to TCDD.

Sex differentiation of liver enzymes has been shown to be controlled by the hypothalamo-pituitary axis [13, 14]. Thus, hypophysectomy leads to abolition of sex differences in drug and steroid metabolism [13]. A striking feature was the fact that TCDD administration induced a liver enzyme pattern displaying less sex differentiation than in uninduced rats. This may indicate that at least some of the effects of TCDD did not result from a direct interaction between the drug and the liver but rather from an action of the drug on the hypothalamo-pituitary axis. TCDD has been shown to increase serum TSH levels in humans 4–5-fold [15] and preliminary observations in our own laboratory indicate that serum levels of prolactin and FSH in rats are affected following treatment with TCDD. In view of these considerations, it seems essential to elucidate the influence of TCDD on the function of the hypothalamo-pituitary axis and the possible consequences of such an influence on liver drug and steroid metabolism.

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