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ALTERED BLOOD LEVELS OF CORTICOSTEROIDS IN THE RAT AFTER EXPOSURE TO 2,3,7,8-TETRACHLORODIBENZO-p-DIOXIN

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2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD), a contaminant produced during the synthesis of chlorinated phenoxyacetic acids, is believed to be one of the most toxic chemicals known, having LD_{50} values of only 1 μg/kg in the guinea pig [1] and 60 μg/kg in the adult male rat [2]. Exposure to TCDD is not characterized by immediate death, but by a delayed lethality of variable length of typically 9-27 days [1]. The previous use of herbicides and waste oil sludges for dust control that have been contamined with TCDD has caused concern about potential health hazards associated with release of this substance into the environment. Adverse effects that have been reported in various mammalian species following exposure to TCDD include diminished food intake [3], chloracne [4], weight loss [3], teratogenesis [5], porphyria [6,7], hepatotoxicity [8] and alterations of parameters of heme synthesis and levels and activities of the hemoprotein cytochromes P-450 in the liver and extrahepatic organs [9-17]. Reports of hirsutism, alopecia, testicular hypoplasia and impaired spermatogenesis after exposure to dioxins [18-24] have led us to investigate interactions of TCDD with the function of endocrine organs. Furthermore, the possibility of accumulation of ^{14}C-labeled TCDD in the adrenal gland [25-27], various reports of pathological lesions in this organ [28,29], and the adverse effects of anorexia, weight loss and hypoglycemia [3,30], suggesting alterations of adrenal insufficiency, have prompted us to determine whether blood levels of corticosteroids might be altered following exposure to this toxicant.

MATERIALS AND METHODS

TCDD (25 μg/kg) was administered in a single, oral dose to adult, male Sprague-Dawley rats (Sasco, Inc., Omaha, NE; 160-180 g). The vehicle, acetone-corn oil (1:2, 2.5 ml/kg), was administered to control rats. Rats were permitted food and water *ad lib.* and were maintained on a controlled lighting cycle (6:00 a.m. lights on; 6:00 p.m. lights off). Rats were decapitated at 2:00 p.m., blood was collected in heparinized tubes and centrifuged, and the plasma was analyzed with a competitive binding radioassay for corticosterone [31]. Third-trimester human plasma was used as the source of the steroid binding protein. The corticosteroids in the methylene chloride extract of rat blood plasma samples were also chromatographed on Silica gel F using benzene-methyl alcohol-water (50:25:25; by vol.) utilizing standards in order to verify adequate separation of steroids and selective measurement of corticosterone.

The zone corresponding to the unknown steroid was eluted twice with 10 ml portions of dichloromethane-ethanol (99:1; v/v). The solvent was evaporated under a stream of nitrogen, and the sample was derivatized as the methoximetrimethylsilyl ether (MO-TMS) by reaction with methoxyamine-HCl in pyridine [32]. The derivatized samples were dissolved in n-hexane and analyzed with a MS-902 mass spectrometer (AEI, Ltd., Manchester, U.K., electron

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impact mode), using a source temperature of 210° and an electron beam energy of 50 eV.

RESULTS AND DISCUSSION

Blood levels of corticosterone were found to be significantly lower than those of controls at days 14 and 21 (Table 1). At day 14, an unknown substance was noticed in the chromatograms from blood samples of TCDD-treated rats with the steroid assay method utilized [3]. The unknown was identified as 11-β-hydroxyprogesterone (Table 2).

Table 1. Rat plasma corticosterone levels following administration of 2,3,7,8-tetrachlorodibenzo-p-dioxin*

Day	Plasma corticosterone (μg/dl)		% of Control
	Control	TCDD	
14	3.2 ± 0.8 (8)	0.9 ± 0.1† (8)	29
21	2.0 ± 0.1 (3)	0.5 ± 0.3† (4)	26

*TCDD was administered in a single, oral dose (25 μg/kg) at day 0. The numbers in parentheses denote the number of rats.

†The difference between TCDD-treated and control values was statistically significant ($P < 0.01$).

Table 2. High resolution electron impact mass spectrometry of an unknown corticosteroid derivative detected in rat blood plasma following exposure to TCDD*

Description of major peaks	Elemental composition	Calculated mass	Reference		Unknown	
			Error (mmu)	Relative intensity	Error (mmu)	Relative intensity
Molecular ion (M+)	$C_{26}H_{44}N_2O_3Si$	460.3119	5.3	79.5	4.7	53.3
M+–15, loss of CH_3	$C_{25}H_{41}N_2O_3Si$	445.2889	0.6	5.4	8.6	7.1
M+–31, loss of $-OCH_3$	$C_{25}H_{41}N_2O_2Si$	429.2935	-1.5	44.3	3.4	38.6
M+–90, loss of $(CH_3)_3SiOH$	$C_{22}H_{34}N_2O_2$	370.2618	3.3	57.8	-2.7	46.6
M+–99, loss of $CH_3C(-CHCH_2)=NOCH_3$	$C_{21}H_{35}NO_2Si$	361.2435	-0.9	24.3	-3.3	22.0
M+–121, loss of $-OCH_3$ and $(CH_3)_3SiOH$	$C_{22}H_{31}N_2O$	339.2434	-2.1	100.00	6.7	100.0

* The sample of blood plasma was obtained from rats at day 21 following exposure to a single, oral dose (25 μg/kg) of TCDD. Measured masses were within 5 millimass units (mmu) of values corresponding to the elemental compositions shown.

The alterations of rat corticosterone blood levels following exposure to TCDD suggest that this toxicant might be interfering with steroidogenesis in the adrenal gland. The isolation of an unknown steroid metabolite from the blood of rats exposed to TCDD, and its identification as 11-β-hydroxyprogesterone, indicate that TCDD produces a block at the 21-hydroxylase step (Fig. 1). The accumulation of 11-β-hydroxyprogesterone is not unexpected. Progesterone has been reported to undergo 11-β-hydroxylation in the rat [33] more readily in

the absence of the normal substrate for the 11-hydroxylase, 11-deoxycorticosterone, as depicted in Fig. 1.

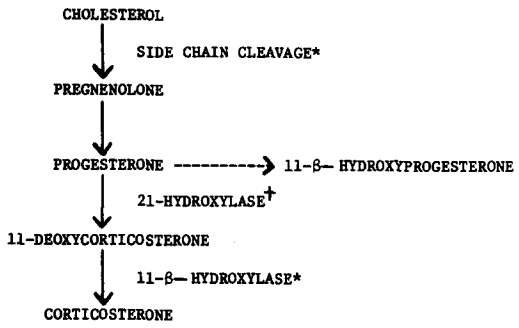

Fig. 1. Pathway of adrenal steroidogenesis in the rat, depicting cytochrome P-450-mediated reactions: cholesterol side-chain cleavage, 21-hydroxylation, and 11-β-hydroxylation. (*) mitochondrial; (†) microsomal.

Other investigators [34] have reported that blood levels of glucocorticoids in the rat were elevated above controls at days 7 and 14 following a single, oral dose (50 μg/kg of TCDD. Because blood levels of glucocorticoids were elevated after observation of various adverse effects associated with exposure to TCDD, the authors conclude that the toxicity of this agent in rats was not mediated by a process involving adrenal hyperfunction. However, no attempt was made to use procedures that were capable of specific measurement of corticosterone nor were hormone values compared to those of controls assayed at the same time periods. The method used merely measured total plasma fluorescence, which would nonspecifically measure a wide variety of fluorescent adrenocortical steroid hormone derivatives. Thus, any steroid derivative accumulating as the result of a block of corticosteroid biosynthesis would be anticipated to inappropriately lead to an erroneous conclusion of elevated levels of corticosterone at later time periods following exposure to TCDD.

The results reported herein suggest that development of adrenal insufficiency associated with depressed corticosterone blood levels may be related to the delayed lethality associated with exposure of rats to TCDD. Rats that were exposed to TCDD, for the study described herein, typically displayed the signs of chronic, diminished food intake and progressive weight loss, characteristic symptoms known to be associated with adrenal insufficiency. It is now apparent that the accumulation of 11-β-hydroxyprogesterone, a corticosteroid derivative not normally found in appreciable quantity in the rat, must also be considered with respect to the adverse effects associated with TCDD in the rat. Furthermore, species variations by which adrenal corticosteroid hormone biosynthesis is regulated may be of importance in our understanding of the proposed species differences in susceptibility to the adverse effects associated with exposure to TCDD. Our laboratory is presently investigating such relationships of corticosteroid hormone biosynthesis to the toxicity associated with exposure to TCDD.

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