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RAPID COMMUNICATION

DECREASED RAT ADRENAL 21-HYDROXYLASE ACTIVITY ASSOCIATED WITH DECREASED ADRENAL MICROSOMAL CYTOCHROME P-450 AFTER EXPOSURE TO 2,3,7,8-TETRACHLORODIBENZO-p-DIOXIN

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2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) is a contaminant generated in the production of phenoxyherbicides. TCDD is believed to be one of the most toxic chemicals known having an LD_{50} value of 1 μg/kg in the guinea pig [1] and 60 μg/kg in the adult male rat [2]. Animals treated with TCDD will typically exhibit reduced food intake and waste away in a starvation-like manner, with death occurring from 1 to 6 weeks after administration of a single oral dose [3,4]. Human exposure to TCDD from spraying of contaminated waste oil sludges for dust control has caused concern about potential health hazards associated with this compound. TCDD-induced hirsutism, alopecia, testicular hypoplasia, impaired spermatogenesis and decreased testosterone production [5-12] suggest toxic effects on endocrine systems. The adverse effects of anorexia, weight loss and hypoglycemia [3,13], as well as the possible accumulation of ^{14}C-labeled TCDD in the adrenal gland [14-16], suggest alterations in adrenal function. Furthermore, previous research in this laboratory has indicated decreased concentrations of serum corticosterone accompanied by accumulation of 11β-hydroxyprogesterone in rats exposed to TCDD [17]. Progesterone has been reported to undergo 11β-hydroxylation in the rat [18] more readily when there are insufficient quantities of the normal substrate for 11β-hydroxylase, 11-deoxycorticosterone. Since 11-deoxycorticosterone is formed by the 21-hydroxylation of progesterone, the presumed absence of 11-deoxycorticosterone suggests a block in the 21-hydroxylase enzyme. Therefore, this study was undertaken to examine the activity of adrenal 21-hydroxylase in the rat following exposure to TCDD. Since 21-hydroxylase is an adrenal microsomal cytochrome P-450-dependent enzyme, and TCDD has been reported to cause decreases in testicular microsomal cytochrome P-450 [19], the concentration of adrenal microsomal cytochrome P-450 was also determined.

MATERIALS AND METHODS

TCDD (50 μg/kg) was administered in a single, oral dose to adult, male Sprague-Dawley rats (Sasco, Inc., Omaha, NE; 220-240 g). The vehicle, acetone-corn oil (1:2, 3.0 ml/kg), was administered to control rats. Rats were permitted food (Purina Rodent Chow, Ralston-Purina Co., St. Louis, MO) 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 killed (8:00 a.m.) by decapitation, and the adrenal glands were removed, trimmed of adhering fat, and weighed. The adrenal glands from two animals were pooled and homogenized in 0.25 M sucrose using a motor-driven glass Potter-Elvehjem homogenizer and a Teflon pestle (0.15 mm clearance). The homogenate was centrifuged at 14,000 g for 15 min to pellet unbroken cells, nuclei and mitochondria. The postmitochondrial supernatant fraction was centrifuged at 105,000 g for 60 min to pellet the microsomes. The microsomal pellet was resuspended in

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buffer (0.1 M KH_2PO_4-glycerol, 4:1, v/v; pH 7.4).

21-Hydroxylase enzyme activity was determined as follows: 200 µl of microsomal suspension (approximately 50 µg protein) was incubated with 500 µM NADPH, 50 mM glucose-6-phosphate (Sigma Chemical Co., St. Louis, MO), 5 units glucose-6-phosphate dehydrogenase (Boehringer Mannheim Biochemicals, Indianapolis, IN), 5 mM $MgCl_2$, and 25 µM (0.56 µCi) [^{14}C]-progesterone (Amersham, Arlington Heights, IL) in a total volume of 0.4 ml. Incubations were conducted in a metabolic shaker at 37° for 10 min. The reaction was terminated by the addition of 1.6 ml methanol at 5°. The deoxycorticosterone formed was isolated by thin-layer chromatography (cyclohexane:ethyl acetate, 45:55, v/v), removed from the plate, and quantified by counting each sample for radioactivity in a Beckman LS8000 liquid scintillation counter.

Concentrations of cytochrome P-450 were determined by the carbon monoxide difference spectrum (450-490 nm) of dithionite-reduced microsomes using a millimolar extinction coefficient of 91 mM^{-1} cm^{-1} [20].

The method of Bradford [21], with bovine serum albumin as the standard, was used for the measurement of protein.

RESULTS AND DISCUSSION

The activity of adrenal 21-hydroxylase was found to be significantly lower than control at days 7 and 14 following the administration of TCDD (Fig. 1). The activity of 21-hydroxylase was decreased to 65% of control on day 7, while day 14 values were decreased to 59% of control. The concentration of adrenal microsomal cytochrome P-450 was decreased significantly at days 7 and 14 after exposure to TCDD (Fig. 2). Adrenal microsomal cytochrome P-450 concentrations were decreased to 72 and 62% of control on days 7 and 14 respectively.

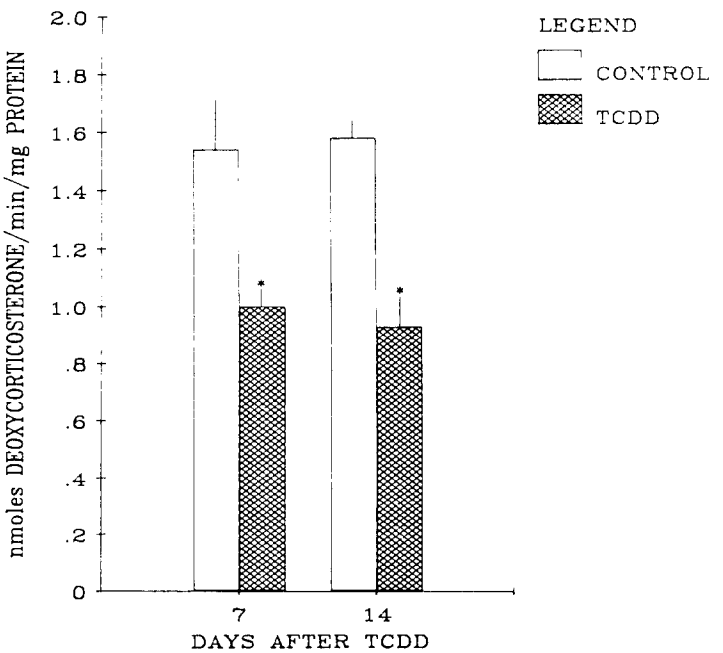

Fig. 1. Effect of TCDD on rat adrenal 21-hydroxylase activity. TCDD (50 µg/kg) was administered orally at day 0. Each bar represents the mean ± SEM for four determinations. *Denotes significant difference ($P < 0.05$) between TCDD-treated and control rats.

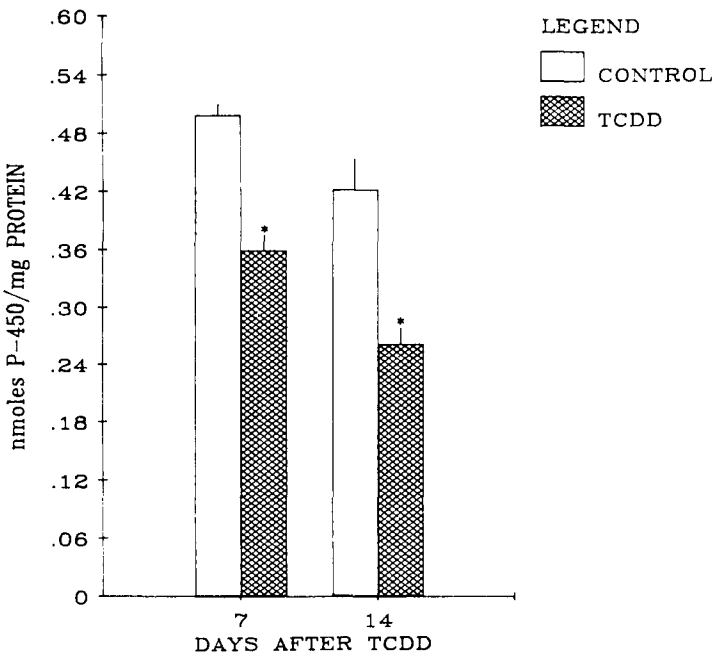

Fig. 2. Effect of TCDD on rat adrenal microsomal cytochrome P-450. TCDD (50 μg/kg) was administered orally at day 0. Each bar represents the mean ± SEM for four determinations. *Denotes significant difference ($P < 0.05$) between TCDD-treated and control rats.

The results reported herein suggest that exposure of rats to TCDD decreases adrenal 21-hydroxylation. The decreases in the activity of 21-hydroxylase are similar to the decreases observed in the concentration of adrenal microsomal cytochrome P-450. Time-dependent decreases have been observed in rat serum corticosterone concentrations [17]. These data indicate that the adrenal insufficiency observed in TCDD-treated rats could be partially due to the decrease in 21-hydroxylase activity. These data are supported by earlier research in this laboratory indicating the accumulation of 11β-hydroxyprogesterone in TCDD-treated rats [17], which could result from an inhibition of adrenal 21-hydroxylase activity [18]. Rats exposed to TCDD typically display signs of hypoglycemia, diminished food intake, and progressive weight loss, characteristic symptoms known to be associated with adrenal insufficiency. Therefore, further research in this laboratory will investigate the relationship of corticosteroid hormone biosynthesis to the toxicity associated with exposure to TCDD.

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