ALTERATIONS IN THYROID FUNCTION IN HOLTZMAN RATS FOLLOWING GESTATIONAL AND LACTATIONAL EXPOSURE TO 2,3,7,8-TETRACHLORODIBENZO-*p*-DIOXIN

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Introduction

2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) induces a variety of toxic responses, including immunotoxicity, teratogenicity and carcinogenicity (1). Thyroid has been identified as one of the target organs of TCDD. Exposure to TCDD has been reported to cause an alteration in thyroid hormone metabolism in the rodent (2, 3). Thyroid hormones play a very important role in brain development during the perinatal period (4). The embryonic stage of development is highly susceptible to TCDD toxicity (5, 6). Recently, growing attention has been directed to the toxicological influence on thyroid of the newborns, especially of human infants, following maternal exposure to TCDD. However, to our knowledge, few studies have been reported how perinatal exposure to TCDD affects pup thyroid although there is a study showing minor effects on thyroxin (T4) levels in Sprague-Dawley rats (7). In addition, there are considerably large variations in susceptibility to TCDD toxicity among rat strains. One of our earlier papers has shown that Sprague-Dawley rats displayed the lowest response among strains tested while Holtzman rats were highly responsive to TCDD in terms of CYP1A1 mRNA induction (8). The aim of the present study was to evaluate the influence of gestational and lactational exposure of TCDD on pup thyroid using the Holtzman rat strain. Thyroid hormone levels were measured in the serum from pups at different developmental stages. The expression of UDP-glucuronosyltransferase (UGT1) mRNA was measured since UGT-1 is believed to be involved in the mechanism of decreased serum T4 level following TCDD exposure (9).

Materials and Methods

Animals and treatments: Pregnant Holtzman rats (5 per group) were given a single oral dose of 200 or 800 ng TCDD/kg on gestational day (GD) 15, or equivalent volume of corn oil as the control. The pups were sacrificed on postnatal days (PND) 21 and 49.

Sample collection and processing: Tissue and blood specimens were collected from 5 infant rats (one per litter) of each group on PND 21and 49. The blood was centrifuged at 900 x *g* for 15 min and the plasma was stored at -80°C until hormone analyses. Portions of the liver were frozen in liquid N_2 and then stored at -80°C until mRNA measurements. Thyroid and pituitary glands were harvested, and then fixed in Zamboni's fixative followed by embedding in paraffin. Tissue sections

were subjected to immunohistochemical staining for T4 and TSH as well as to morphological evaluations.

Thyroid hormone analysis: Serum levels of thyroxin (T4) and triiodothyronine (T3) were determined using radioimmunoassay (RIA) kits (Amerlex-M: Amersham LIFE SCIENCE). Thyroid stimulating hormone (TSH) was determined using enzyme immunoassay (EIA) system (rTSH EIA system, Amersham LIFE SCIENCE).

CytochromeP4501A1 (CYP1A1) and UGT-1 mRNA analysis: Induction of TCDD-responsive genes, CYP1A1 and UGT-1, in the pup liver was analyzed by mRNA quantification using RT-PCR (2).

Immunohistochemical detection of T4, PCNA and TSH: T4 and PCNA in thyroid and TSH in pituitary were visualized according to method described in our previous paper (10).

TCDD analysis: Livers, adipose tissues and serum from the male pups were analyzed for TCDD concentration on PND 21 and 49 by using gas chromatography-mass spectrometry procedures with specific ion monitoring (10).

Statistics: Values are expressed as the mean ± SEM. One-way ANOVA was employed to assess dose effects and *post hoc* comparisons were made by Fisher's PLSD test.

Results and Discussion

The TCDD analysis showed that almost the same amounts of TCDD were accumulated in the liver and adipose tissue of pups on PND 21, containing 2000 pg/g tissue in liver of an 800 ng TCDD/kg dose group. However, on PND 49, the amount of hepatic TCDD was dramatically decreased to approximately 2 % of that of 21-day-old pup. While gene expression of CYP1A1 was markedly induced in the liver of TCDD-exposed rats both on PND 21 and 49, the level of UGT1 gene by TCDD was significantly induced on PND 21, but decreased to control levels by PND 49, in consistent with a marked reduction of hepatic TCDD content. Serum T4 levels in both male and female pups were decreased significantly by exposure to TCDD on PND 21 in the 200 and 800 μg/kg dose groups, but, interestingly, restoration or even significant increase in T4 levels were observed on PND 49 in the both dose groups. A significant increase in circulating T3 was also found in the pups exposed at 800 ng TCDD /kg. A dose of 800 ng TCDD/kg resulted in greater than a 2-fold increase in serum TSH levels in male pups on PND 21, and this increased levels continued until PND 49 even when circulating T4 levels reached more than those of the control. The stimulatory effects of TCDD on T4 and TSH biosynthesis were confirmed by immunocytochemical examination. We showed that TCDD treatment resulted in an increase not only in intensity of immunostaining of thyroid hormones, but also in number of TSH-positive cells in target organs. In histological examinations, the gestational and lactational exposure at 800 ng TCDD/kg resulted in hyperplastic lesion of follicular cells in thyroid glands of pups on PND 49. A quantification of hyperplastic changes in the pup thyroid by measuring the ratio of parenchymal area to follicular area revealed a significant increase in the ratio in response to TCDD. Proliferating cell nuclear antigen (PCNA) immunocytochemistry also supported this proliferative lesion of follicular cells by TCDD exposure. It has been well-established that circulating T4 and T3 are regulated by TSH at the levels of the hypothalamus-pituitary- thyroid axis under physiological conditions. It seems likely that a decreased levels of circulating T4 observed on PND 21 was due to enhanced excretion of T4-glucuronide by TCDD-induced hepatic UGT-1 as shown in the present study. There is no explanation for the unexpected increase in both T4 and TSH levels on PND 49. Sustained excretion of TSH resulted in hyperplastic lesion in thyroid as evidenced by morphometric evaluation and PCNA immunohistochemistry. It seems thus reasonable to postulate that this abnormality of thyroid hormone homeostasis is attributable to

disorder of a feedback control system at step(s) on the hypothalamus-pituitary-thyroid axis.
Although possible explanation is not provided at present, it is of interest to note that hyperplasia is produced preferentially in male rather than in female pups.
Based on the experimental evidence from the present study, we would conclude that perinatal exposure to even low doses of TCDD could disturb thyroid hormone homeostasis including a sustained excessive secretion of TSH, leading to an irreversible hyperplasia of follicular cell in pup thyroid.

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