Expression of dioxin -related genes in response to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) in various tissues of Long Evans rats

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Abstract

Expression of CYP1A1, CYP1A2 and Ah receptor mRNA, induction of CYP1A1 and Ah receptor protein in brain, liver, spleen, thymus of male and female Long Evans rats in response to 100, 300 and 1000 ng TCDD/kg body weight were investigated. In addition, mRNA expression of CYP1A1, CYP1A2 and Ah receptor in testes, prostate and uterus were also investigated. Sex different expression of CYP1A1 mRNA, CYP1A2 mRNA and Ah receptor protein was observed in liver. Tissue specific expression of CYP1A2 mRNA and Ah receptor mRNA in testes and prostate were also observed.

Introduction

Most, if not all, of the effects of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) and related compounds are mediated through binding to a cytosolic protein, known as aryl hydrocarbon receptor (AhR). Interaction of TCDD with AhR is considered to be the initial step that ultimately results in altered gene expression and the observed toxic response (1-3). AhR is a ligand-activated transcription factor, which forms a heterodimer with the aryl hydrocarbon receptor nuclear translocator (ARNT). This heterodimer interacts with dioxin responsive enhancer elements (DRE) upstream of the target genes and activate their transcription (4, 5). Induction of CYP1A1 gene expression, mediated by the Ah receptor, is considered to be an early and sensitive biochemical response and can be used as a marker for exposure or responsiveness to TCDD (6, 7).

It is well known that the responsiveness to toxic effects of dioxins varies with sex, tissues and species. Difference in Ah receptor mediated gene expression might be involved in these variations. However, there were few reports in experimental animals investigating the expression of Ah receptor mediated genes in response to TCDD in various tissues in both sexes.

In the present study, expression of CYP1A1, CYP1A2 and Ah receptor mRNA, CYP1A1 and Ah receptor protein in brain, liver, spleen, thymus of rats in response to various doses of TCDD were investigated. In addition, mRNA expression of CYP1A1, CYP1A2 and Ah receptor in testes, prostate and uterus were also investigated since endocrine disrupting properties of dioxin have attracted great concern. Results from the present study will provide basal information

involved in different sensitivity to TCDD among tissues and between sexes.

Experimental Methods

Animals and treatment

Six-week old male and female Long Evans rats (Charles River Japan) were administered TCDD (Cambridge Isotope Laboratory, MI, USA) in corn oil with a single oral dose of 100, 300, 1000 ng/kg body weight. After 7 days, animals were sacrificed and tissues were excised, quickly frozen in liquid nitrogen and subsequently stored at -80°C.

RT-PCR analysis

Total RNA was extracted using Isogen (Nippon Gene, Japan) according to the manufacturer's instructions. RT-PCR was performed with a RT-PCR kit (Takara Biomedical, Japan). PCR primers were designed using OLIGO 5 software (National Biosciences, Plymouth, USA) and sequence information available for SD rat through the National Center for Biotechnology Information (National Library of Medicine, USA). The PCR reaction was carried out in a final volume of 50 μl using a Perkin Elmer Gene Amp PCR system 2400. PCR condition for CYP1A1, CYP1A2 and cyclophilin was the same. PCR products were separated in 1.5% agarose gel, visualized with ethidium bromide staining and photographed.

Protein detection

Cytosolic and microsomal fraction of tissues were prepared according to the method of Lu and Levin with little modification (8). CYP1A1 protein in microsomal fraction and AhR protein in cytosol were separated by SDS-polyacrylamide gel electrophoresis and detected by Western immunoblotting using enhanced chemiluminescence (ECL kit, Amersham).

Results and Discussion

Expression of CYP1A1 mRNA and CYP1A2 mRNA in brain, liver, spleen, thymus of male and female rats in response to 100, 300 and 1000 ng TCDD/kg body weight were shown in Fig. 1 (A). In both sexes, CYP1A1 mRNA was expressed dose-dependently in all the tissues examined while CYP1A2 mRNA was expressed only in liver (all dose levels) and spleen (1000 ng/kg). As CYP1A2 is proposed to be a microsomal binding protein of TCDD (9), distribution of TCDD in brain and thymus was expected to be much less than spleen and liver.

In liver, CYP1A1 and CYP1A2 mRNA were constitutively expressed and the levels were higher in females than in males. CYP1A1 protein, dose-dependently expressed only in liver, was higher in females than in males (data not shown). Furthermore, there existed marked sex difference in Ah receptor protein expression in liver. Ah receptor protein was expressed in female rats while only faint signal of Ah receptor protein was detected at 1000 ng TCDD/kg body weight treated male rats (data not shown). Sex difference in CYP1A1 and CYP1A2 mRNA expression, CYP1A1 protein and Ah receptor protein expression in the liver might be relevant to the gender-specific response to TCDD such as liver carcinogenesis in Sprague-Dawley rats (10).

Expression of Ah receptor mRNA in thymus, spleen, liver and brain of untreated male and female rats were shown in Fig. 1(B). Clear signals of Ah receptor mRNA were detected only in liver of both sexes.

As in utero and lactational exposure of very low dose of TCDD reduced sperm number in rats and long-term low-dose exposure of TCDD to rhesus monkeys developed endometriosis, Ah receptor mediated gene expression in genital tissues are of great concern.

Expression of CYP1A1, CYP1A2 mRNA in testes, prostate and uterus in response to 100, 300 and 1000 ng TCDD/kg body weight were shown in Fig. 2 (A). Expression of Ah receptor

mRNA in testes, prostate and uterus of untreated rats were shown in Fig. 2(B). CYP1A1 mRNA and CYP1A2 mRNA were expressed in prostate but only CYP1A1 mRNA was expressed in testes of rats treated with 1000 ng TCDD/kg body weight . Ah receptor mRNA was expressed only in prostate but not in testes. No mRNA expression of CYP1A1, CYP1A2 and Ah receptor was observed in uterus. As Ah receptor mediated the most of the biological effects of TCDD and CYP1A2 is proposed to be a microsomal binding protein of TCDD in the liver as mentioned

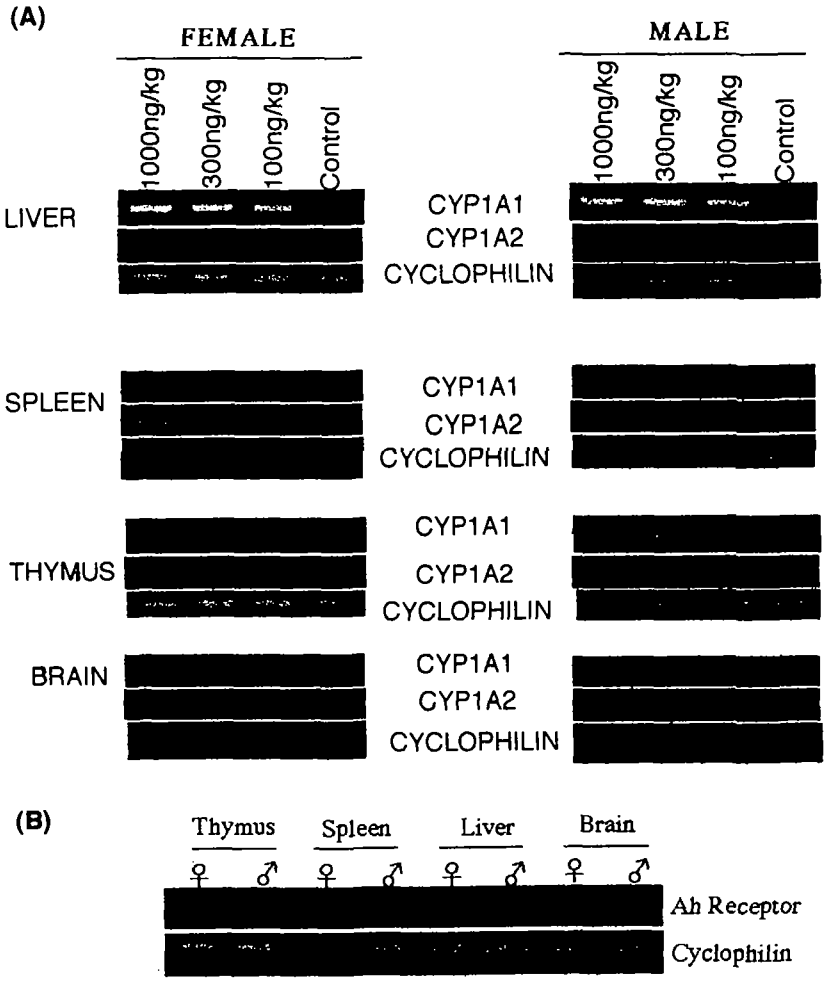

Fig. 1. (**A**) Expression of CYP1A1 and CYP1A2 mRNA in response to various doses of TCDD in liver, spleen, thymus and brain of male and female Long Evans rat.
(**B**) Expression of Ah receptor mRNA in thymus, spleen, liver and brain of control male and female Long Evans rats.

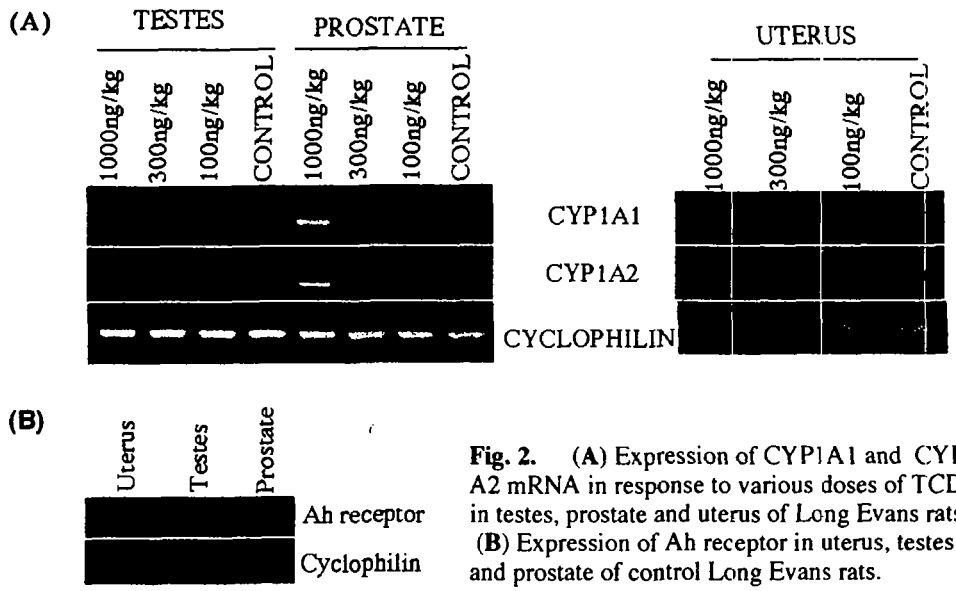

Fig. 2. (**A**) Expression of CYP1A1 and CYP1 A2 mRNA in response to various doses of TCDD in testes, prostate and uterus of Long Evans rats. (**B**) Expression of Ah receptor in uterus, testes and prostate of control Long Evans rats.

above (9), responsiveness to TCDD might differ between testes and prostate. Ejaculated sperm numbers and cauda epididymal sperm numbers were reduced by 45% and 18%, respectively while testicular sperm numbers were not affected in rats offspring injected with 0.8 μg TCDD/kg once at gestation day 15 (11). These results suggested the different responsiveness to toxic effects of TCDD among male genital tissues. Difference in expression of dioxin-related genes among male genital tissues in response to TCDD need further investigation to elucidate the mechanism involved in tissue specific responsiveness.

In summary, (a) sex different expression of CYP1A1 mRNA, CYP1A2 mRNA and Ah receptor protein in liver and (b)tissue specific expression of CYP1A2 mRNA and Ah receptor mRNA between testes and prostate were observed.

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