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Dioxin-induced retardation of development through a reduction in the expression of pituitary hormones and possible involvement of an aryl hydrocarbon receptor in this defect: A comparative study using two strains of mice with different sensitivities to dioxin

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ABSTRACT

We have previously revealed that treating pregnant rats with 2,3,7,8-tetracholorodibenzo-*p*-dioxin (TCDD) reduces the expression of gonadotropins and growth hormone (GH) in the fetal and neonatal pituitary. A change in gonadotropin expression impairs the testicular expression of steroidogenic proteins in perinatal pups, and imprint defects in sexual behavior after reaching maturity. In this study, we examined whether TCDD also affects the expression of gonadotropin and GH in mice using C57BL/6J and DBA/2J strains which express the aryl hydrocarbon receptor (Ahr) exhibiting a different affinity for TCDD. When pregnant C57BL/6J mice at gestational day (GD) 12 were given oral TCDD (0.2–20 μg/kg), all doses significantly attenuated the pituitary expression of gonadotropin mRNAs in fetuses at GD18. On the other hand, in DBA/2J mice, a much higher dose of TCDD (20 μg/kg) was needed to produce a significant attenuation. Such reduction in the C57BL/6J strain continued until at least postnatal day (PND) 4. In agreement with this, TCDD reduced the testicular expression of steroidogenic proteins in C57BL/6J neonates at PND2 and 4, although the same did not occur in the fetal testis and ovary. Furthermore, TCDD reduced the perinatal expression of GH, litter size and the body weight of newborn pups only in the C57BL/6J strain. These results suggest that 1) also in mice, maternal exposure to TCDD attenuates gonadotropin-regulated steroidogenesis and GH expression leading to the impairment of pup development and sexual immaturity; and 2) Ahr activation during the late fetal and early postnatal stages is required for these defects.

Introduction

2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD), the most toxic of the series of chemicals classified as dioxins and related compounds, is a representative environmental pollutant, and its harmful effects on the health of humans and wildlife have long been of great concern. It is well known that dioxins cause a number of toxic effects, such as wasting syndrome, carcinogenesis, immunosuppression and teratogenicity (Poland and Knutson, 1982). Many these effects, if not all, are believed to take place following the activation of a nuclear receptor, the aryl hydrocarbon receptor (AhR) (Fernandez-Salguero et al., 1996; Mimura et al., 1997). The complex of AhR and dioxin migrates to the nucleus, and binds to its cognitive sequence, designated as the xenobiotic responsive element (XRE), present in the 5′-upstream region of the target genes to change the gene expression (Fujisawa-Sehara et al., 1987; Mimura and Fujii-Kuriyama, 2003). Although more than 200 genes are affected by the AhR:XRE-dependent mechanism (Frueh et al., 2001), the target genes defining the toxic effects of dioxins remain poorly understood. In addition, developmental disorders including growth retardation and sexual immaturity occur in pups whose mothers were exposed to dioxins at lower doses during the perinatal stages (Bjerke et al., 1994; Gray et al., 1995, 1997; Huuskonen et al., 1994; Mably et al., 1992). This is potentially serious because the dioxin dose needed to cause these effects is far lower than those for acute toxicity, and the abnormalities produced may be carried over from one generation to another (Peterson et al., 1993). Several human studies also suspect that maternal exposure to dioxins and other AhR agonists is associated with developmental disorders including sexual immaturity in born children (Grandjean et al., 2012; Guo et al., 2000; Tsukimori et al., 2013).

Abbreviations: αGSU, glycoprotein hormone α-subunit; Ahr, aryl hydrocarbon receptor; Cyp, cytochrome P450; EGF, epidermal growth factor; FSH, follicle-stimulating hormone; GD, gestational day; GH, growth hormone; GnRH, gonadotropin-releasing hormone; GnRH-R, GnRH receptor; HSD, hydroxysteroid dehydrogenase; IGF1, insulin-like growth factor 1; LH, luteinizing hormone; PND, postnatal day; RT-PCR, reverse transcription-polymerase chain reaction; StAR, steroidogenic acute-regulatory protein; TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; TSH, thyroid-stimulating hormone; XRE, xenobiotic responsive element.

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Thus, much more research focusing on the TCDD-induced retardation of pup growth is urgently required to draw up measures for the protection of child health.

Our previous studies have revealed that treating pregnant rats with TCDD at gestational day (GD) 15 reduces the pituitary expression of gonadotropins [luteinizing hormone (LH) and follicle-stimulating hormone (FSH)] during the fetal and neonatal stages, leading to the attenuated expression of steroidogenic proteins including steroidogenic acute-regulatory protein (StAR) and cytochrome P450 (CYP) 17 in their gonads (Mutoh et al., 2006; Takeda et al., 2009, 2012; Taketoh et al., 2007). Direct supplementation of equine chorionic gonadotropin, an LH-mimicking hormone, into the fetuses exposed to TCDD at GD15 restored not only the attenuated expression of gonadal steroidogenic proteins but also the defects in sexual behavior after reaching maturity (Takeda et al., 2009, 2014). These observations strongly suggest that a TCDD-produced reduction in gonadotropin biosynthesis in fetal rats triggers the disruption of gonadal steroidogenesis, and imprints defects in sexual behavior after they reach maturity. In addition, our recent study indicated that maternal exposure to TCDD suppressed the pituitary production of growth hormone (GH) in fetal and neonatal rats (Hattori et al., in press). This change also seems to be linked to developmental disorders. Indeed, a TCDD dose-dependent attenuation in fetal body weight took place concomitantly with the lowered expression of pituitary GH. Thus, the research focusing on the relation between a reduction in GH expression and the growth disturbance of fetuses and neonates appears to be important for a better understanding of TCDD effects on the next generation. However, the following fundamental questions remain to be answered: 1) whether the TCDD-induced lowering of gonadotropin-regulated steroidogenesis and GH expression occurs ubiquitously in species other than rats, and 2) whether Ahr activation in perinatal pups also contributes to these disorders.

Pituitary primordial cells in both rats and mice are gradually differentiated to the five cell types including gonadotropes, LH/FSH-producing cells, from around GD12 by stimulation with cell type-specific transcriptional factors (Begeot et al., 1982; Borrelli et al., 1989; Scully and Rosenfeld, 2002). Then, LH synthesis in gonadotropes starts at GD16 (Aubert et al., 1985; Japón et al., 1994). However, while substantial stimulation by LH of testicular steroidogenesis does not work in mice until the early neonatal stage (O'Shaughnessy et al., 1998; El-Gehani et al., 1998; George et al., 1978; Poling and Kauffman, 2012), LH-dependent steroidogenesis in male rats already emerges from the late fetal period, GD19.5 (Habert and Picon, 1982; Migrenne et al., 2001). It has long been considered that either the exposure (males) or its absence (females) of developing brain to pup's own sex-steroids is necessary for acquiring gender-specific phenotypes such as sexual behavior (Carlson, 2007; McEwen, 1981). To generate masculine phenotypes, the stimulation by sex-steroids supplied from the testis must take place during a narrow window of the perinatal stage designated as the 'critical period' (Goy and McEwen, 1980; MacLusky and Naftolin, 1981). In agreement with the species difference mentioned above in terms of the time when LH-assisted steroidogenesis becomes active, the critical period appears to differ between rats and mice: i.e., while the critical period in rats ranges from late fetal stage to neonatal stage, that in mice begins after birth (MacLusky and Naftolin, 1981). Conceivably, such species difference may contribute to the species variety of disorders caused by fetal exposure to dioxin.

In view of the above pieces of information and background, we examined here whether gonadotropin-regulated steroidogenesis and GH expression are disrupted by TCDD in mouse fetuses and neonates. The present study examined this issue using two strains of mouse; i.e., C57BL/6J and DBA/2J. It is well known that these mice have different sensitivity to dioxins due to the expression of functionally-divergent Ahrs. That is, C57BL/6J and DBA/2J mice have an Ahr exhibiting a high and low affinity for dioxins, respectively (Chapman and Schiller, 1985; Poland et al., 1974). Thus, we also investigated the contribution of Ahr activation to the TCDD-damaged expression of pituitary hormones and gonadal steroidogenic proteins by comparing TCDD responsiveness between both strains of mice.

Materials and methods

Materials

TCDD was purchased from Accu Standard Inc. (New Haven, CT). A rabbit antibody against rat StAR and mouse anti-β-actin monoclonal antibody were purchased from Santa Cruz Biotechnology Inc. (Dallas, TX) and BioVision Inc. (Mountain View, CA), respectively. All other reagents were of the highest grade commercially available.

Animals and treatments

All animal experiments were conducted under the approval of the Institutional Animal Care and Experiment Committee of Kyushu University. C57BL/6J and DBA/2J mice (6 weeks old) were purchased from CLEA Japan Inc. (Tokyo, Japan). They were housed in an air-conditioned and specific pathogen-free room where the photoperiod was set to be a 12 h light/12 h dark cycle, and food and tap water were provided ad libitum. After adaptation for a week, female mice were paired overnight with male mice. When a seminal plug was observed in the female vagina, the day was designated as GD 0 of pregnancy. In all experiments, pregnant mice at GD12, the age when primordial cells in the anterior pituitary begin to differentiate to hormone-producing cells, were given a single oral dose of TCDD (0.2, 1, 5 and 20 μg/kg/5 mL corn oil) or vehicle. Based on the assumption that the development of the pituitary-gonadal axis and brain sexual differentiation in mice occurs during the postnatal stage (O'Shaughnessy et al., 1998), the tissues were removed from not only their fetuses at GD18 but also neonates at postnatal day (PND) 2, 4 and 7. The TCDD dose was set on the basis of our previous study reporting that maternal exposure to 1 μg/kg TCDD reduces the expression of pituitary gonadotropins in perinatal rat pups (Takeda et al., 2012). Because it is conceivable that sensitivity to TCDD differs between mice and rats, we examined here the effect of several doses ranging from lower to higher than 1 μg/kg. However, a higher TCDD dose (5 μg/kg) was lethal for the majority of neonates in the C57BL/6J strain (see the Results section for the details).

Reverse transcription-polymerase chain reaction (RT-PCR)

The expression of mRNAs was quantified by real-time RT-PCR according to the method described elsewhere (Matsumoto et al., 2010). In brief, total RNA was extracted from the testis, ovary, pituitary and hypothalamus using an RNeasy Mini Kit (QIAGEN GmbH, Hilden, Germany). The RNA (100–250 ng) obtained was treated with gDNA Eraser for digestion of contaminated genomic DNA, and reverse-transcribed to its cDNA, using a PrimeScript® RT reagent kit (Takara-bio Inc., Shiga, Japan). The cDNAs transcribed from target mRNAs were amplified with Fast SYBR Green Master Mix (Life Technologies, Carlsbad, CA), using a StepOnePlus Real-time PCR system (Life Technologies). The primer sequences are shown in the Supplemental Table 1. The PCR conditions were as follows: 95 °C, 20 s–40 cycles (95 °C, 3 s–60 °C, 30 s). Each relative expression of mRNAs was determined using standard curve method. The amount of quantified target mRNA was normalized by β-actin mRNA, and it was shown as a ratio to the control.

Immunoblotting

The expression of testicular StAR and β-actin proteins was analyzed by immunoblotting according to the method described previously (Takeda et al., 2009). The testes were removed from fetuses and neonates during GD18 and PND7, and those from 2–5 pups/one dam were