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Perinatal TCDD exposure alters developmental neuroendocrine system

R.G. Ahmed

Division of Anatomy and Embryology, Zoology Department, Faculty of Science, Beni-Suef University, Egypt

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ABSTRACT

This study tested whether maternal exposure to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) may disrupt the development of neuroendocrine system of their offspring during the perinatal period. TCDD (0.2 or 0.4 μg/kg body weight) was orally administered to pregnant rats from gestation day (GD) 1 to lactation day (LD) 30. Potential effects on neuroendocrine function were evaluated by measuring serum thyroid hormone levels in pregnant rats and their offspring and measuring some biochemical parameters in cerebellum of these offspring on GD 16 and 19, and LD 10, 20, and 30. In both treated groups, a decrease in serum thyroxine (T4), triiodothyronine (T3) and increase in thyrotropin (TSH) levels were noticed during the tested days in dams and offspring, as well as GH levels were decreased in offspring with respect to control group. In cerebellum of control offspring, the levels of monoamines, γ-aminobutyric acid (GABA) and acetylcholinesterase (AchE) were found to be increased from GD 16 to LD 30. The hypothyroid conditions due to both maternal administrations of TCDD produced inhibitory effects on monoamines and AchE, and stimulatory actions on GABA in cerebellum of offspring. These alterations were dose and age dependent. Overall, these results suggest that TCDD may act as neuroendocrine disruptor.

1. Introduction

2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) is a potent environmental and developmental toxicant (Tanida et al., 2009; Darnerud et al., 2010; Goodman et al., 2010; Matsumoto et al., 2010). It belongs to polychlorinated aromatic hydrocarbons (PAHs) group, which include polychlorinated biphenyls (PCBs), polychlorinated dibenzofurans (PCDFs) and polychlorinated dibenzo-*p*-dioxins (PCDDs) (Koibuchi, 2006; Hennig et al., 2007; Nishijo et al., 2007; Brouillette and Quirion, 2008; Crofton, 2008; Darras, 2008; Sul et al., 2009; Tanida et al., 2009; Darnerud et al., 2010; Goodman et al., 2010; Matsumoto et al., 2010). These organohalogens are bioaccumulated and biomagnified in the food chain, meat and milk products (including breast milk) (Hassoun et al., 2000; Darnerud et al., 2010), and in the tissues of wildlife, domestic and marine animals and human's worldwide (Gilbert, 2003; Ishida et al., 2005; Charnley and Kimbrough, 2006; Hennig et al., 2007; Brouillette and Quirion, 2008; Smith et al., 2008; Tanida et al., 2009). Particularly, TCDD is an unintentional by-product of multiple anthropogenic processes such as bleaching using chlorine gas, combustion and incineration of wastes (municipal, hospital, hazardous), fabrication of pesticides and herbicides, wood products, tobacco smoke, production of iron and steel, coal-fired electric power generation, photochemical and enzymatic reactions in sewage sludge (Viluksela et al., 2004; Hood et al., 2006; Smith et al., 2008; Tanida et al., 2009; Darnerud et al., 2010). TCDD has a long half-life in animals (Byers et al., 2006) and in humans (approximately 8 years) (Byers et al., 2006; Goodman et al., 2010).

Due to the high lipophilicity and relatively slow metabolism of TCDD, this compound accumulates in maternal fat stores and cross the placenta (Kreuzer et al., 1997; Mori, 2001; Petersen et al., 2006; Nishijo et al., 2007; Tanida et al., 2009) to produce a wide variety of toxic effects in offspring (Kuchiiwa et al., 2002) as a permanent brain damage (Petersen et al., 2006). Dioxin affects the development of offspring during embryonic and fetal periods even when the exposure level is too low to induce toxicity in the mother (Mably et al., 1992). In Europe and USA, perinatal exposure to background levels of dioxins cause persistent effects during childhood (ten Tusscher and Koppe 2004). These effects may be related to alterations in thyroid functions (Takser et al., 2005; Wang et al., 2005; ten Tusscher et al., 2007; Leijs et al., 2008; Pearce and Braverman, 2009) which are essential for normal development in utero and in infancy (Bruno et al., 2005; Gilbert and Sui, 2006; Carageorgiou et al., 2007; Ahmed et al., 2008; Koibuchi, 2008; Leonard, 2008; Shibutani et al., 2009; Wirth et al., 2009; Zhang et al., 2009; Davis et al., 2010; Di Paola et al., 2010; Fu et al., 2010; Jugan et al., 2010; Sigrun and Heike, 2010). However, another investigation failed to show associations between dioxins and THs in maternal serum of pregnant women (Foster et al., 2005).

THs are strongly involved in vertebrate brain development, from early embryogenesis to subsequent prenatal and perinatal development particularly in mammals (Bruno et al., 2005; Gilbert and Sui, 2006; Carageorgiou et al., 2007; Ahmed et al., 2008; El-bakry et al., 2010; Horn and Heuer, 2010; Jugan et al., 2010).

E-mail addresses: ahmedragab08@gmail.com, r_g_a_ahmed@yahoo.com

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Also, the developing brain is very sensitive to TCDD exposure (Markowski et al., 2002; Kakeyama and Tohyama, 2003; Kim et al., 2007) where the maternal exposure delayed the fetal brain growth and neurodevelopment in offspring of rat (Nishijo et al., 2007) and of non-human primates (Schantz et al., 1992; Negishi et al., 2006). These alterations might cause subtle but profound and irreversible deficits in brain functions in adulthood (Negishi et al., 2006). The interference mechanism of persistent organic pollutants (dioxin) with THs metabolism in the mother, fetus and newborn may injure the developing brain (Porterfield, 2000; Winneke et al., 2002; ten Tusscher and Koppe, 2004; Roelens et al., 2005; Grandjean and Landrigan, 2006; Brouillette and Quirion, 2008), even at background environmental levels (Darras, 2008), via the availability of TH transporters (Friesema et al., 2005, 2010; Heuer et al., 2005; Nunez et al., 2008). Also, several hypotheses linking the mechanisms of TCDD neurotoxicity with the alterations of central monoaminergic, glutamatergic and cholinergic systems have been proposed. TCDD exposure (Pohjanvirta and Tuomisto, 1994) and THs dysfunction (Mason et al., 1990) may alter various neurotransmitter systems (Hassoun et al., 2000), particularly biogenic amines (Porterfield, 2000). More so, PCB may alter γ-aminobutyric acid (GABA)-mediated pathways (Bushnell and Rice, 1999) and may decrease the activity of choline acetyltransferase (ChAT) (Juarez de Ku et al., 1994) in the developing brain. These observations are the same as those seen in neonatally hypothyroid rats (Porterfield, 2000). However, these mechanisms are not fully understood.

Unfortunately, little is known about the neurodevelopment of dioxins, despite their impact on society. Thus, the present study aims to determine whether the exposure of pregnant white albino rats (*Rattus norvegicus*, Wistar strain) to TCDD through pre- and post-natal periods may disturb the development of neuroendocrine system, particularly the interactions between THs and development of cerebellum. Notably, TCDD was examined herein because it is the prototype dioxin-like chemical and the most potent congener (Hassoun et al., 2000). Also, rat cerebellum was used as a model system, because this region is highly sensitive to any stress (TH disturbance) during perinatal period (Koibuchi and Chin, 2000; Li et al., 2004; Yousefi et al., 2005; Ahmed et al., 2008; Koibuchi, 2009).

2. Materials and methods

2.1. Chemicals

2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD; purity >99%), corn oil, norepinephrine (NE), epinephrine (E), dopamine (DA), serotonin (5-HT), γ-aminobutyric acid (GABA), thiocholiniodide, 5,5′-dithiobis-2-nitrobenzoic acid (DTNB), acetylthiocholiniodide, sodium tartrate, copper tartrate, trichloroacetic acid (TCA) and general chemicals were purchased from Sigma Chemical Company (Sigma, St. Louis, MO, USA). Thyroxine (T4), triiodothyronine (T3) and thyrotropin (TSH) kits were obtained from Diagnostic Products Corporation (DPC) (Los Angeles, USA), as well as growth hormone (GH) kit was purchased from BioSource Europe S.A. (Belgium).

2.2. Experimental animals

Mature white albino rats (*Rattus norvegicus*, Wistar strain) were purchased from the National Institute of Ophthalmology, Giza, Egypt. This study was carried out on 18 mature virgin females/group weighing about 170–190 g and 9 mature males/group for mating only. They were kept under observation in the department animal house for 2 weeks to exclude any intercurrent infection and to acclimatize the new conditions. The animals were marked, housed in metal (stainless steel; 60 × 50 × 50 cm) separate bottom ventilated cages at normal atmospheric temperature (23 ± 2 °C) and fed on standard rodent pellet diet manufactured by the Egyptian Company for oil and soap as well as some vegetables as a source of vitamins (Ahmed et al., 2007b, 2010; El-bakry et al., 2010). Tap water was used for drinking ad libitum and these animals were exposed to constant daily light/dark periods of 12 h each (lights on at 06:00 h) and 50 ± 5% relative humidity (Ahmed, 2009; Ahmed et al., 2010; El-bakry et al., 2010). All animal procedures are in accordance with the general guidelines of animal care and the recommendations of the Canadian Committee for Care and use of animals (Canadian Council on Animal Care, 1993). All efforts were made to minimize the number of animals used and their suffering.

Daily examination of vaginal smears of each virgin female was carried out to determine the estrus cycle. Estrous females exhibited the presence of cornified cells in vaginal smears. Mating was induced by housing proestrus females with male in separate cage at ratio of two females and one male overnight for one or two consecutive days. In the next morning, the presence of sperm in vaginal smears determined the first day of gestation. Then, the pregnant females were transferred into separate cages from males to start the experiment.

2.3. Experimental strategy

Non-anesthetized pregnant rats received two doses of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) (0.2 or 0.4 μg/kg body weight) orally by gastric intubation and daily from gestation day (GD) 1 to lactation day (LD) 30 (between 7.30 and 8.30 a.m.). TCDD was dissolved in corn oil vehicle being a highly lipophilic compound (Hassoun et al., 2000; Darnerud et al., 2010). Oral administration of TCDD is the most likely route of entry into the animal in natural conditions and the doses were selected based on preliminary dose–response studies. TCDD (0.2 μg/kg) was the concentration giving the lowest adverse effect observed while 0.4 μg/kg TCDD was the highest dose giving adverse effect (no obvious signs of developmental toxicity). Corn oil vehicle only was orally administered to control group by gastric intubations. Dams and their offspring were decapitated under mild diethyl ether anesthesia and sampled at GD 16 and 19, and at LD 10, 20, and 30.

The mother blood samples (6 per group) were taken from jugular vein during the gestational period at day 16 and 19 and lactational period at day 10, 20 and 30. Fetal blood samples (6 per group) were collected directly from the umbilical cord at GD 16 and 19 while the pup's blood samples were taken from jugular vein at postnatal day (PND) 10, 20 and 30. The clotted blood samples were centrifuged at speed 3000 rpm (1006.2*g*) and at temperature 15–24 °C for 30 min. The clear, non-hemolysed supernatant sera were quickly removed, divided into three portions for each individual animal, and kept at −30 °C until use for different hormonal assays (radioimmunoassay). On the other hand, cerebellum of rat offspring was quickly removed, separated and homogenized by using a Teflon homogenizer (Glas-Col, Terre Haute in USA), and kept in deep freezer at −30 °C until use for different developmental and biochemical assays.

2.4. The radioimmunoassay examinations

T4, T3 and TSH in serum of mothers and their offspring, as well as GH in serum of their offspring only were estimated according to the method of Thakur et al. (1997), Maes et al. (1997), Mandel et al. (1993) and Reutens (1995), respectively.

2.5. The developmental and biochemical examinations in cerebellum of offspring

2.5.1. High performance liquid chromatography (HPLC) analysis

2.5.1.1. Estimation of monoamines concentrations. The monoamines concentrations were estimated according to the method of Pagel et al. (2000). Cerebellum was homogenized in 75% aqueous HPLC grade methanol. The homogenates were spun at 3000 rpm for 15 min and the supernatants were immediately extracted from the trace elements and lipids by the use of solid phase extraction CHROMABOND column NH2 phase Cat. No. 730031. The samples were then injected directly to the AQUA column (150 × 4.6 mm, 5μ and C18) (phenomenex, USA) under the following conditions: mobile phase 97/3 20 mM potassium phosphate, pH 3.0/methanol, flow rate 1.5 ml/min, UV 270 nm. Additionally, norepinephrine, epinephrine, dopamine and serotonin were separated after 12 min, the resulting chromatogram for each sample identified each monoamines position and area under curve was compared to that of the standard curve made by Eurochrom HPLC Software, version 1.6. Calculation: Concentration of sample (μg/g) = Concentration of standard (μg/ml) × volume of homogenization/weight of tissue (g) × area of sample under curve/area of standard under curve.

2.5.1.2. Estimation of γ-aminobutyric acid (GABA) concentration. GABA concentration was determined according to the method of Chakrabarti and Poddar (1989). A 10% (w/v) homogenates were prepared in 0.25 M cold sucrose. Protein free filtrates of cerebellum homogenates were prepared by mixing the homogenates with equal volumes of 10% TCA. This was followed by centrifugation in cold at 3000 rpm for 15 min. GABA content was measured using sodium tartrate and copper tartrate, respectively, after developing fluorophores by ninhydrin with the protein-free filtrate. Calculation: Concentration of sample (μg/g) = Concentration of standard (μg/ml) × volume of homogenization/weight of tissue (g) × area of sample under curve/area of standard under curve.

2.5.2. Estimation of acetylcholinesterase (AchE) activity

The method used in this study was a modification of Ellman method (Ellman, 1978) using acetylthiocholiniodide as substrate. Add 0.1 ml homogenate in the assay system [0.15 ml phosphate buffer (20 mM, pH 7.6) and 0.05 ml substrate (0.1 M acetyl-thiocholiniodide)]. Then, the reaction was stopped by 1.8 ml