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Endocrine-Disrupting Chemicals and Human Growth and Maturation: A Focus on Early Critical Windows of Exposure

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Abstract

Endocrine-disrupting chemicals (EDCs) are exogenous substances that interfere with hormone synthesis, metabolism, or action. In addition, some of them could cause epi-genetic alterations of DNA that can be transmitted to the following generations. Because the developing organism is highly dependent on sex steroids and thyroid hormones for its maturation, the fetus and the child are very sensitive to any alteration of their hormonal environment. An additional concern about that early period of life comes from the shaping of the homeostatic mechanisms that takes place also at that time with involvement of epigenetic mechanisms along with the concept of fetal origin of health and disease. In this chapter, we will review the studies reporting effects of EDCs on human development. Using a translational approach, we will review animal studies that can shed light on some mechanisms of action of EDCs on the developing organism. We will focus on the major hormone-dependent stages of development: fetal growth, sexual differentiation, puberty, brain development, and energy balance. We will also discuss the possible epigenetic effects of EDCs on human development.

1. INTRODUCTION

Endocrine-disrupting chemicals (EDCs) are exogenous substances that interfere with hormone synthesis, metabolism, or action. Moreover, it appears that some of them could cause epigenetic alterations of the DNA that can be transmitted to the following generations. Animal and human studies have brought evidence that EDCs affect male and female reproduction, thyroid function, and control of energy balance. They could increase the risk of breast or prostate cancer as well as the risk of metabolic syndrome (Diamanti-Kandarakis et al., 2009). Because the developing organism is highly dependent on sex steroids and thyroid hormones for its maturation, the fetus and the child are very sensitive to any alteration of their hormonal environment. An additional concern about that early period of life comes from the shaping of the homeostatic mechanisms that takes place also at that time with involvement of epigenetic mechanisms along with the concept of fetal origin of health and disease (Gluckman, Hanson, & Low, 2011). Most studies have identified the perinatal period as a specific window of sensitivity. However, most of the reported effects were observed later in life. A review of the existing literature underlines the need for identification of early markers of exposure to EDCs. In this chapter, we will review the studies reporting effects of EDCs on human development. Using a translational approach, we will review animal studies that can shed light on some mechanisms of action of EDCs on the developing organism. We will focus on the major hormone-dependent stages of development: fetal growth, sexual differentiation, puberty, brain development, and energy balance. We will also discuss the possible epigenetic effects of EDCs on human development.

2. CHALLENGES IN EVIDENCING ENDOCRINE DISRUPTION

Before we discuss the different aspects of growth and maturation that are possibly altered by endocrine disruption, it is important to be aware of some challenges (Table 1.1) that we face in this area and that are relevant to all the specific aspects we will discuss later. Because the persistence of EDCs in the body and the environment is highly variable between few days such as for bisphenol A (BPA) (Rudel et al., 2011) and several decades such as for1,1-dichloro-2,2-bis (p-chlorophenyl) ethane (DDE) (Kirman, Aylward, Hays, Krishnan, & Nong, 2011), linking any disorder with previous EDC exposure is most difficult especially when latency is long between exposure and manifestation of health consequences. Also, the effects of EDCs can vary depending on the critical periods and duration of exposure. As will be discussed in the next sections, prenatal and early postnatal life is a period characterized by organization of the mechanisms that will drive homeostatic processes such as control of reproduction and energy balance. Obviously, EDC interference during those organizing periods could have much more severe consequences than later in life. Among the features of endocrine systems, they involve a cascade of activation or inhibition at different levels where EDCs play disturbing roles. We will see illustrations with puberty and reproduction that can be altered by effects at the hypothalamic-pituitary level as well as in target tissues (e.g., breasts). This also applies to energy balance through involvement of hypothalamic centers as well as fat tissue. Moreover, the physiological feedback systems through factors such as

sex steroids and leptin, respectively, will also be disturbed by EDCs. Further challenges come from observations that are inconsistent with classical toxicology: Low-dose mixtures that are consistent with human exposure can have effects not conforming to simple additive models (Christiansen et al., 2012; Kortenkamp, 2008); the dose—response relationship can be nonmonotonic such as seen for BPA with U-shaped dose—response curves (Vandenberg et al., 2012). For both reasons, setting a threshold dose for EDC effects has become meaningless. A final issue is the highly variable latency between exposure and effects including multigenerational impact.

Table 1.1 Challenges in the demonstration of endocrine disruption

1	Variable persistence in the body and the environment
2	Variable effects depending on the critical periods and duration of exposure
3	Simultaneous action at different interrelated levels of endocrine systems
4	Low-dose mixtures consistent with human exposure not conforming to simple additive models
5	Nonmonotonic dose-response relationship
6	Variable latency between exposure and effects including multigenerational impact

3. ENDOCRINE-DISRUPTING CHEMICALS AND FETAL GROWTH

Data concerning EDCs effects on fetal growth are scarce. However, one can hypothesize that fetal growth could be altered by endocrine disruption. Indeed, several EDCs cross the placental barrier and accumulate in the embryo or amniotic fluid (Diamanti-Kandarakis et al., 2009). The fetus is particularly sensitive to the effects of EDCs because of its dependency on hormones for development (Diamanti-Kandarakis et al., 2009). Moreover, animal studies have shown that most biotransformation enzymes are not produced until after birth (Pottenger et al., 2000), which means that fetuses might be exposed longer to higher concentration of EDCs. Clearance of BPA from fetal circulation, for instance, is slower than from maternal circulation (Takahashi & Oishi, 2000). It remains very complex to evaluate the effects of prenatal exposure to EDCs on fetal growth in human. Most studies focus on correlations between birth weight and serum or urinary levels of EDCs during pregnancy or at birth. Because of some limitations discussed later, few studies have identified a link between prenatal exposure to EDCs and fetal growth. We will review here some of the most significant human data as well as supporting animal studies.

For BPA, for instance, few studies have been published and lead to various results. Miao et al. have shown that maternal exposure to BPA in the workplace was associated with decreased birth weight (Miao et al., 2011b) after adjusting for confounding factors. BPA exposure during pregnancy was evaluated through personal air-sampling measurements and exposure history. Chou et al. (2011) reported an increased risk of low birth weight in male newborn exposed prenatally to higher levels of BPA, while Padmanabhan did not report any effect of BPA neither on birth weight nor on length (Padmanabhan et al., 2008). In both studies, prenatal exposure to BPA was evaluated through a single measurement of BPA in maternal or cord blood. Philippat et al. have shown an association between urinary BPA concentration (in one urinary sample between 24 and 30 weeks of gestation) and birth weight following an inverse U shape (Philippat et al., 2012). However, serial urinary measurements before and during pregnancy have been shown to be highly variable and this variability was even increased during pregnancy (Braun et al., 2011a). Given this variability, it appears that more than one sample may be necessary to adequately classify gestational exposure to BPA especially because the half-life is short and the clearance rate is rather rapid as opposed to other EDCs. Rudel et al. (2011) showed that urinary excretion of BPA fell significantly 2-3 days after changing habits regarding food, drinks, and containers. In addition, for feasibility reasons, most studies focus on one or a few compounds and might miss exposure to other EDCs. Another limitation of current epidemiological studies is the studied parameters: most studies focus on birth weight, while they might oversee more subtle effects of EDCs on body composition.

Some epidemiological studies have identified a negative correlation between exposure to polybrominated diphenyl ethers (PBDEs) and birth weight. PBDEs are flame-retardant chemicals used in the manufacture of infant products, furniture, and electronics. Ninety-seven percent of the American population appears to be contaminated by those persistent EDCs (Sjödin et al., 2008). Animal studies have shown that PBDEs disrupt thyroid function and alter behavior and memory (Herbstman et al., 2010). A prospective study in a population of 286 pregnant women with low income living in California showed that higher concentrations of PBDEs in

maternal serum during pregnancy were associated with lower birth weight. Each 10-fold increase in concentrations of BDE-47, BDE-99, and BDE-100 was associated with a 115 g decrease in birth weight (Harley et al., 2011). Other smaller studies had similarly shown that higher concentrations of PBDEs were associated with a higher risk of delivering lower birth weight infants (Chao, Wang, Lee, Wang, & Päpke, 2007; Wu et al., 2010). Some human studies, however, did not identify any effects of PBDE exposure on birth weight (Mazdai, Dodder, Abernathy, Hites, & Bigsby, 2003; Tan, Loganath, Chong, & Obbard, 2009). Animal models have not reported an effect of PBDEs on birth weight but identified a decreased weight gain of offspring during the postnatal period, which is comparable to the third trimester of pregnancy in human (Kodavanti et al., 2010). The tested doses were however elevated and difficult to translate into relevant environmental exposure.

4. EDCs AND SEXUAL DIFFERENTIATION

Sexual differentiation depends on prenatal hormonal environment.

Therefore, exposure to EDCs may be associated with disorders of development of the reproductive system by altering this hormonal environment. Cryptorchidism and hypospadias in the male newborn and low sperm counts and increased risk of testicular germ cell cancer in young adult males belong to a lifelong spectrum of disorders caused by early impairment of testicular function. This association of disorders has been proposed as the testicular dysgenesis syndrome (TDS) (Skakkebaek, Rajpert-De Meyts, & Main, 2001). TDS appears to involve deficient testosterone (androgen) production by the fetal testis (Sharpe & Shakkebaek, 2008). Indeed, it has been shown that normal development of the male reproductive system depends on the crucial role of androgen within an early fetal time window, called the mas-culinization programming window (Welsh et al., 2008), which influences the reproductive capacity throughout life. Thus, EDCs, which interfere with the synthesis or action of androgens, can have deleterious consequences for the developing male genital tract and appear to be risk factors for TDS (Bay, Asklund, Skakkebaek, & Andersson, 2006).

Several animal experimental studies have confirmed this hypothesis, especially using the phthalates, a group of anti-androgenic compound present in personal care products, coating of pharmaceutical products, and soft plastics. Male offspring of pregnant rats exposed to 250 mg/kg or more of monobenzyl phthalate, a major metabolite of butyl benzyl phthalate, on days 15-17 of pregnancy had an increased incidence of undescended testes and a decreased anogenital distance, a marker of androgenic impregnation (Ema, Miyawaki, Hirose, & Kamata, 2003). Likewise, Fisher et al. reported a TDS-like condition after fetal rat exposure to phthalate esters (Fisher, Macpherson, Marchetti, & Sharpe, 2003). The effects of phthalates on male sexual development in rats result from alterations of Leydig cell function leading to androgen insufficiency, which can be responsible of hypospadias or cryptorchidism. Swan et al., in 2005, have demonstrated a similarly reduced androgenization after phthalate exposure in humans since boys whose mother had elevated prenatal phthalate exposure, measured through phthalate urinary concentration, had shorter anogenital distance and impaired testicular descent (Swan et al., 2005). Main et al. (2006) reported that free testosterone levels at age 1-3 months were negatively correlated with monoethyl phthalate (MEP) levels in breast milk collected during that period. A reduced Leydig cell response to LH was suggested by the increasing LH/free testosterone ratio in relation to milk MEP levels (Main et al., 2006). Though these findings suggest similar mechanisms in both rodents and humans, recent studies indicate possible differences in testicular sensitivity to phthalate effects among humans and rodents. Given the difficulty to evaluate the production of testosterone by the human fetal testis, Mitchell et al. (2012) and Heger et al. (2012) used a xenograft model to evaluate the effects of phthalates on steroidogenesis of human fetal testis. They used second-trimester human fetal testes xenografts, which were exposed to phthalates for 1-4 days or 21 days. There was no difference in serum testosterone levels or in the seminal vesicles weight between xenograft model exposed to DBP or MBP and controls (Mitchell et al., 2012). Heger et al. (2012) reported that steroidogenesis was suppressed in rodent xenograft but not in human xenografts in such conditions. Germ cell alterations however were observed in human xenografts.

Data concerning BPA are controversial. In animal studies, perinatal BPA exposure has been shown to lead to decreased levels of testicular testosterone (Richter et al., 2007) or impaired fertility (Salian, Doshi, & Vanage, 2009). These data are consistent with two human epidemiological studies highlighting the negative effect of BPA on the male reproductive function, by modifying sex hormone concentrations (Galloway et al., 2010; Meeker, Calafat, & Hauser, 2010). However, other animal and human studies have reported conflicting results when studying effect of BPA on male reproductive tract. Indeed, in human, an association between maternal exposure to BPA during pregnancy and a shorter anogenital distance in male offspring has been shown (LaRocca, Boyajian, Brown, Smith, & Hixon, 2011), while cord blood BPA levels are not different between normal and cryptorchid boys (Ema et al., 2001). In rodents, several studies have reported that BPA exposure *in utero* had no effect on the adult male reproductive system (Fénichel et al., 2012; Kobayashi, Ohtani, Kubota, & Miyagawa, 2010; Meeker et al., 2010; Miao et al., 2011a).

Interestingly, one recent study has evaluated the effects of perinatal exposure to BPA and diethylhexyl phthalate on gonadal development of male mice. Using a mixture of EDCs (DEHP and BPA) better illustrates the synergistic effects of a combination of EDCs as encountered in the environment (Xi et al., 2012). Significant reduction in testicular weight and/or epididy-mal sperm count was identified in immature and mature animals on postnatal days 15 and 42. Serum testosterone levels were also decreased. These authors however used doses of EDCs that were higher than those relevant for human exposure and mixture effects.

We discussed earlier the peripheral effects of EDCs on sexual differentiation. However, brain sexual differentiation should not been ignored because of its sensitivity to hormonal environment and its significance for reproduction. It is well established that testosterone secreted by the fetal and neonatal testis is involved in brain sexual differentiation, most likely after it has been converted to estradiol by aromatase in specific brain regions during critical periods of development (Rubin et al., 2006). Thus, perinatal exposure to BPA could alter sex steroid action in the rodent brain and disrupt the development of sexually dimorphic pathways. This concept is confirmed by data available in animals. Kubo et al. demonstrated that BPA exposure during prenatal and postnatal period abolished the sex differences in open-field behavior in mice. Likewise, it increases the size of the locus coeruleus (LC) in males and decreases LC volume in females (Kubo et al., 2003). Rubin et al. also identified an effect of BPA on brain sexual differentiation since exposure to low doses of BPA decreased the sexually dimorphic population of tyrosine hydroxylase (TH) neurons in the rostral periventricular preoptic area, an important brain region for estrous cyclicity and estrogen-positive feedback (Rubin et al., 2006). Similarly, a decreased sexual dimorphism in the number of corticotropin-releasing hormone neurons in the bed nucleus of the stria terminalis has been shown after BPA exposure, whereas there was no effect in the preoptic area (Funabashi, Kawaguchi, Furuta, Fukushima, & Kimura, 2004). Moreover, Tando et al. have shown that BPA exposure can affect brain development in a sex-specific manner. Indeed, a significant reduction in the total volume and density of dopaminergic neurons in the substantia nigra (SN) is observed only in adult female offspring after maternal BPA treatment during pregnancy and lactation (Tando et al., 2007). The cited studies underline the need to focus on both sexes when studying EDCs in order to identify sexual dimorphism in sensitivity to endocrine disruption.

5. EDCs AND PUBERTY

The effects of EDCs on puberty have been investigated mainly through variations in pubertal timing with emphasis on the onset of sexual maturation. Therefore, our current knowledge may miss some additional effects such as changes in age at occurrence of regular (ovulatory) cycles. As stated earlier, the appraisal of EDC effects on puberty is complex due to involvement of possible effects on peripheral target organs such as uterus and breast in females and penis in males as well as effects on neuroendocrine control of maturation through hypothalamic-pituitary maturation. Second, pubertal timing can be influenced by exposure close to the time of puberty or during the process of puberty as well as much earlier in life since pubertal timing is one among the parameters programmed during fetal/neonatal life. The published human observations after exposure that was estimated to have occurred pre- or neonatally are summarized in Table 1.2 and those after postnatal exposure in Table 1.3. The data are scarce and it appears that no firm conclusion can be drawn. It is of note that, except in conditions of accidental massive exposure to a single class of EDCs, mixtures are likely involved in most conditions. Therefore, measurement of particular compounds and study of a compound in relation to pubertal timing may involve some biases.

6. EDCs AND BRAIN DEVELOPMENT

Several studies have reported that prenatal or early postnatal exposure to some EDCs is associated with alterations of cognitive or motor functions in children. Knowing the fundamental role played by thyroid hormones and sex steroids in cortex development, one can hypothesize that disruption of those hormones could cause alteration of the development of the cerebral cortex and of its functions later in life. We will review here the human data suggesting a causal effect for endocrine disrupters on impairment of cortical functions and approach some EDC mechanisms of action using animal models.

Table 1.2 Effect of prenatal or early postnatal exposure to EDCs on timing of breast development and menarche

Pubertal timing	**Early**		**Normal**		**Delayed**	
Event	**Breast**	**Menarche**	**Breast**	**Menarche**	**Breast**	**Menarche**
DDE (+DDT)	Krstevska-Konstantinova et al. (2001)	Vasiliu, Muttineni, and Karmaus (2004)				
PCBs			Vasiliu et al. (2004), Yang et al. (2005)			
Dioxins			Leijs et al. (2008), Warner et al. (2004)		Leijs et al. (2008)	
Mixture of pesticides	Wohlfahrt-Veje et al. (2012)					

Table 1.3 Effect of postnatal exposure to EDCs on timing of breast development and menarche

Pubertal timing	**Early**		**Normal**		**Delayed**
Event	**Breast**	**Menarche**	**Breast**	**Menarche**	**Breast Menarche**
DDE(+DDT)		Ouyang et al. (2005)	Wolff et al. (2008)	Denham et al. (2005)	
PCBs	Denham et al. (2005)	Denham et al. (2005)	Den Hond et al. (2002), Wolff et al. (2008)	Den Hond et al. (2002)	
Dioxins				Den Hond et al. (2002)	Den Hond et al. (2002)
Phthalates	Colon et al. (2000), Wolff et al. (2010)		Lomenick et al. (2010), Frederiksen et al. (2012)		Pubarche: Frederiksen et al. (2012)
Soy phytoestrogens			Strom et al. (2001)	Strom et al. (2001)	Wolff et al. (2008, 2010)

6.1. Disruption of thyroid function and brain development

Thyroid hormones are known to be essential for brain development. They regulate progenitor proliferation and differentiation, neuron migration, and dendrite outgrowth (Parent, Naveau, Gerard, Bourguignon, & Westbrook, 2011). Even mild thyroid hormone insufficiency in humans can produce measurable deficits in cognitive functions (Zoeller & Rovet, 2004). Thyroid hormone action is mediated by two classes of nuclear receptors (Forrest & Vennström, 2000) that exhibit differential spatial and temporal expressions in the brain, suggesting that thyroid hormones have variable functions during brain development (Horn & Heuer, 2010). This differential expression of thyroid hormone receptors explains the critical period of thyroid hormone action on brain development as suggested by models of maternal hypothyroidism or congenital hypothyroidism (Zoeller & Rovet, 2004). Depending on the timing of onset of hypothyroidism, the offspring will display problems of visual attention, gross or fine motor skills, or language and memory skills. Similarly, one can hypothesize that disruption of thyroid function by EDCs will have different effects based on the timing of exposure. However, few studies focused on that aspect.

Polychlorinated biphenyls (PCBs) form a group of widespread environmental contaminants composed of 209

different congeners used in a wide variety of applications. Their production was banned in the 1970s but PCBs are still present in the environment due to their high stability. PCBs were among the first EDCs identified as responsible for alterations of cognitive functions. Indeed, impaired memory and altered learning abilities have been associated with prenatal exposure to EDCs in humans and rodents (Schantz, Widholm, & Rice, 2003). In animal models, perinatal exposure to PCBs has been consistently associated with a decrease of thyroid hormone concentration in maternal serum as well as pup serum (Brouwer et al., 1998). Some but not all epidemiological studies in human have found an association between PCB body burden and thyroid hormone levels (Langer, 2008). This disruption of thyroid function could explain some of the effects of PCBs on the developing brain. Indeed, animal models have shown that the ototoxic effects of PCBs could be partially ameliorated by thyroxin replacement and PCBs seem to alter some of the developmental processes in the cortex and the cerebellum that are dependent on thyroid hormones (Diamanti-Kandarakis et al., 2009). However, recent publications raise important issues. First, PCBs produce paradoxical effects on the thyroid system; PCBs reduce serum T_4 but increase the expression of some genes regulated by TH (Zoeller, Dowling, & Vas, 2000). Second, while some report agonistic actions of PCBs on the TH receptor (Gauger et al., 2007), others report antagonistic actions (Koibuchi & Iwasaki, 2006). Although this appears paradoxical, it is consistent with *in vivo* studies showing that PCBs can exert different actions on TH response genes in the developing brain (Bansal & Zoeller, 2008). In addition, as suggested by *in vitro* models, some congeners can have direct toxic effects on neurons through alterations of neurotransmission or intracellular signaling, independent of disruption of thyroid hormones. PBDEs are semivolatile and migrate into house dust, placing the young children at risk of higher exposure (Stapleton et al., 2008). Animal studies suggest that pre- and postnatal exposure to different PBDE congeners causes long-lasting behavioral alterations, in particular alterations of motor activity and cognitive behavior (reviewed in Costa & Giordano, 2007). As it is the case for other EDCs, some windows of susceptibility have been identified during pre- and postnatal brain development (Eriksson, Viberg, Jakobsson, Orn, & Fredriksson, 2002; Kuriyama & Chahoud, 2004). Recent studies have shown that exposure to PBDEs causes alteration of thyroid hormone levels in pregnant women (Chevrier et al., 2010) and infants (Herbstman et al., 2008) as it is the case in rodents. Only very few studies, however, have focused on the molecular or cellular effects of perinatal exposure to PBDEs *in vivo*. Viberg et al. have reported a decrease of cholinergic nicotinic receptors in the hippocampus after exposure to BDE-99 and BDE-153 (Viberg, Fredriksson, & Eriksson, 2003). However, the link between such a decrease and the behavioral effects of PBDEs is still unclear. Other teams have reported that exposure to PBDEs reduced hippocampal long-term potentiation and decreased brain-derived neurotrophic factor expression in the brain (Viberg, Mundy, & Eriksson, 2008). While several studies have reported negative effect of PBDEs on brain development and cognitive function in animals, there is relatively little information about adverse health effects of PBDEs in humans. Some very recent studies have identified a correlation between prenatal exposure to PBDEs and alteration of cognitive functions. Eskenazi et al. have reported that both prenatal and early postnatal PBDE exposures were associated with poorer attention and fine motor coordination and cognition in a cohort of 300 school-age children at 5 and 7 years of age (Eskenazi et al., 2012).

6.2. Disruption of sex steroid action and brain development

Sex steroids also play a major role during brain development. Androgens and estrogens sculpt the gender-specific differences of brain regions involved in behavior, learning, memory, mood, and socialization. Both androgens and estrogens stimulate progenitor proliferation in the cortex and hippocampus. However, it has been shown in the hippocampus that androgens preferably support neurogenesis, whereas estrogens promote gliogenesis (Zhang, Konkle, Zup, & McCarthy, 2008). Estrogens and aromatizable androgens also regulate dendritic outgrowth, synaptic function, and neuronal connectivity. Because several EDCs affect estrogen and androgen receptors directly or indirectly through an effect on sex steroid biosynthesis, it is important to examine the effects of those EDCs on development of the cerebral cortex, the hippocampus, and the hypothalamus. As an illustration, we will summarize here some of the effects of BPA on behavior and cognitive functions in animals and humans. As developed earlier in this chapter, BPA has been shown to alter sexually dimorphic behaviors such as aggression, anxiety, and exploration in rodents (Palanza, Gioiosa, vom Saal, & Parmigiani, 2008; vom Saal et al., 2007). Sexually dimorphic disorders such as autism, attention deficit, or hyperactivity could be correlates of these animal behaviors and might be related to early exposure to EDCs. Braun et al. (2011a) studied a prospective cohort of 244 mothers and their 3-year-old children. BPA exposure was evaluated by measuring BPA levels in maternal urine at 16 and 26 weeks of gestation as well as BPA urinary levels in the children at 1, 2, and 3 years of age. Each 10-fold increase in gestational BPA exposure was associated with more anxious and depressed behavior and poorer emotional control in the 3-year-old children (Braun et al., 2011b). Effects appeared to be sexually different since girls, for example, exhibited increased hyperactivity, while boys exhibited decreased hyperactivity after gestational exposure to BPA. It is not known however if those effects will persist later in life. Those results illustrate the importance of the window of exposure since behavior appeared to be affected by prenatal exposure to BPA but not by postnatal exposure.

One can hypothesize that perinatal exposure to EDCs could lead to alterations of the development of brain circuits and an increased risk of neu-rodevelopmental deficits. Indeed, the developing brain is remarkably malleable. Such plasticity is advantageous in that it allows the refinement of the basic organization in response to the surrounding environment. However, this plasticity also can be maladaptive in that these critical developmental periods are extremely vulnerable to disruption as illustrated by neurodevelopmental disorders such as autism or fetal alcohol syndrome. Such diseases often are characterized by a disruption of functional brain circuits in the setting of grossly normal brain morphology. Animal models could be used to identify cellular or molecular markers of EDCs effects. Indeed, the synapse is the fundamental unit responsible for formation of brain circuitry and thus should serve as a sensitive indicator of disruption by EDCs. Leranth et al. have shown that adult exposure to BPA prevents the synaptogenic response to estradiol in hippocampus and prefrontal cortex in ovariectomized nonhuman primates (Leranth et al., 2008a) and antagonizes spine formation induced by estrogens and testosterone in limbic areas in gonadectomized female and male rats (Leranth et al., 2008b; MacLusky et al., 2005). Those data were obtained after BPA exposure during adult life. A more recent study, however, showed that gestational exposure to BPA reduced the number of dopamine neurons in the midbrain and the number of spine synapses in the hippocampus, while no effects were observed when the animals were exposed during the juvenile period (Elsworth et al., 2013).

7. EDC AND ENERGY BALANCE

The concept of developmental or fetal origin of adult disease was developed by Barker at the end of the 1980s. He showed that nutritional status during early life is linked to an increased risk of cardiovascular disease in early-adult life and premature death as a consequence (Barker & Osmond, 1986; Barker, 2004). Several studies initially focused on the correlation between fetal growth (often assessed by the birth weight) and the predisposition to adult disease, in particular metabolic disorders. Thereby, it has been shown that low birth weight is correlated with a major risk to develop insulin resistance (Hales et al., 1991), metabolic syndrome (Barker et al., 1993), and obesity (Ravelli, Van Der Meulen, Osmond, Barker, & Bleker, 1999) during adulthood.

The possible implication of EDCs during pregnancy in the development of diseases in adulthood was first evocated in the early 1970s, after millions of pregnant women were prescribed DES (diethylstilbestrol). Indeed, daughters of woman treated by DES, a pharmaceutical estrogen given to prevent miscarriages, had an increased incidence of vaginal adenocarcinoma and benign reproductive lesions (Herbst, Ulfelder, & Poskanzer, 1971).

All these data highlight intrauterine and early postnatal life as critical periods for future health. According to this idea, one can hypothesize that the significant increase of obesity and metabolic syndrome incidence might be linked to perinatal disrupting factors acting as predisposition factors. Exposure to EDCs perinatally, by altering hormonal environment, could be associated with disorders of energy balance throughout subsequent life.

This hypothesis has been evocated in several animal studies. Significant data have been obtained for DES and BPA. Howdeshell et al. showed that pregnant mice fed with BPA (2.4 μg/kg/day on 11-17 days of gestation) had heavier pups than control mice (Howdeshell, Hotchkiss, Thayer, Vandenbergh, & vom Saal, 1999). This finding was confirmed in 2001, by Rubin, who exposed pregnant rat to BPA (0.1 mg BPA/kg/day or 1.2 mg BPA/kg/day) and observed an increase in body weight of the male and female offspring, an increase that was more persistent in females than males (Rubin et al., 2001). BPA has also been shown to reduce glucose tolerance and increase insulin resistance in male offspring at 6 months of age (Alonso-Magdalena et al., 2010). Interestingly, some studies suggest that these changes in glucose regulation are persistent in adult offspring and are worsened if the offspring is fed with a high-fat diet (Wei et al., 2011). The data of Ryan et al., however, are not consistent with this findings since they have shown that BPA exposure during pregnancy (0.25 μg/kg/day) results in accelerated growth early in life but does not result in impaired glucose regulation in adulthood, even when the mice are maintained on a high-fat diet (Ryan et al., 2010).

In addition to altering glucose tolerance, BPA appears to affect adipogenesis very early in life. Female pups born from dams exposed to 1 mg/L of BPA in drinking water during gestation and lactation showed adipocyte hypertrophy and overexpression of lipogenic genes such PPAR-γ, SREBP-IC, SCD-1, and C/EBP-ALPHA (Somm et al., 2009). Masuno et al., using 3T3-L1 cells, a preadipocyte cell line that differentiates into mature adipocytes, have shown that BPA leads to an accelerated differentiation into adipocytes, causing high accumulation of triglycerides and lipoprotein lipase (Masuno, Iwanami, Kidani, Sakayama, & Honda, 2005; Wang, Sun, Hou, Pan, & Li, 2012). More recently, the effect of BPA on adipogenesis has been confirmed by Sargis et al., who assessed the ability of BPA (100 nM) to activate the glucocorticoid receptor and thereby increase lipid accumulation and expression of adipocytic proteins in mature adipocytes (Sargis, Johnson, Choudhuryl, & Bradyl, 2010).

In addition, exposure to BPA, at 10 nM, 1 μM, and 80 μM, increased the mRNA expression and enzymatic activity of 11β-HSD1, an enzyme that converts the inactive hormone cortisone to the active hormone Cortisol in adipose tissues and promotes adipogenesis (Masuno et al., 2002). Interestingly, BPA at low doses (1 and 10 nM) also decreases adiponectin production from human adipose tissue (Hugo et al., 2008). Adiponectin is an adipocyte-specific hormone that increases insulin sensitivity and reduces tissue inflammation (Whitehead, Richards, Hickman, Macdonald, & Prins, 2006). Similarly, it has been shown that release of IL-6 and TNF alpha, two inflammatory cytokines involved to obesity, is stimulated by BPA exposure (Ben-Jonathan, Hugo, & Brandebourg, 2009).

Effects of BPA on energy balance are also suggested by studies in humans. A large cross-sectional study in human, by Lang et al., has shown that higher urinary BPA concentrations in adults were associated with diagnosis of cardiovascular disease and diabetes and abnormal concentrations of three liver enzymes (gamma-glutamyltransferase (GGT), alkaline phosphatase, and lactate dehydrogenase) (Lang et al., 2008; Vom Saal & Myers, 2008). However, measurements of BPA were made in adults and do not bring any information about the critical perinatal period identified in animals. A recent study in children showed that incidence of obesity was increased among children who had higher urinary BPA concentrations (Trasande, Attina, & Blustein, 2012). However, it is difficult to correlate BPA exposure and BPA urinary excretion. Moreover, the causal link remains difficult to establish. Indeed, obese children could, for instance, consume more BPA-contaminated food such as canned sodas, which would explain their higher urinary BPA levels. Further longitudinal studies are thus necessary in humans in order to determine if gestational and postnatal exposure to BPA could lead to an increased risk of metabolic syndrome later in life.

8. EPIGENETIC PERSPECTIVE ON THE DEVELOPMENTAL EFFECTS OF EDCs

Epigenetics refers to heritable alterations that are not due to changes in DNA sequence. Rather, epigenetic modifications, such as DNA methyla-tion and histone modification, alter DNA accessibility and chromatin structure, thereby regulating patterns of gene expression. This mechanism appears as an adaptive response to insults during the developmental period, such as variations in maternal diet or sex steroids (Fowden & Forhead, 2009; Lillycrop et al., 2007). For example, it has been recently proposed that maternal undernutrition could cause epigenetic changes in the proopiomelanocortin (POMC) and glucocorticoid receptor genes in the fetal hypothalamus (Stevens, Begum, & White, 2011). POMC-derived peptides synthesized in neurons of the hypothalamus play a central role in the control of energy homeostasis (Coll, Sadaf, Chains, Yeo, & O'Rahylly, 2004).

EDCs, by interfering with the nutritional and/or hormonal environment during fetal life, may interfere with epigenetic programming, which can be passed from one cell generation to another and persist in adulthood (MClachlan, 2001; Anway, Cupp, Uzumcu, & Skinner, 2005). Moreover, epigenetic modifications, when they occur in the gonads, can be transmitted to the next generations as illustrated by Anway et al. (2005).

Some studies have reported the effects of prenatal BPA exposure on epigenetic mechanisms. Dolinoy et al., using the Agouti viable yellow mouse model, have shown that BPA exposure during pregnancy led to DNA hypomethylation of the offspring epigenome. Interestingly, this effect can be counteracted by maternal dietary supplementation (diet enriched with methyl group donors) (Dolinoy, Huang, & Jirtle, 2007). It will be necessary to examine the correlation between BPA-induced epigenetic alterations, modification in gene expression, and phenotype expression. But the absence of hypomethylation when supplementing maternal dietary during pregnancy suggests possible means for reducing risk of disease. Other studies supported epigenetic effects of BPA. Bromer et al. have shown that *in utero* exposure to high doses of BPA increased the expression of the homeobox gene Hoxa 10 in the uterus of female offspring at 2 weeks of age. This was correlated with significant demethylation of specific CpG sites in the Hoxa 10 gene, a gene necessary for uterus development. It is interesting to note that Hoxa 10 DNA methylation was not altered in adult mice treated with these doses of BPA. It highlights the great vulnerability of the fetus and the existence of a critical developmental window for the epigenetic effects of EDCs (Bromer, Zhou, Taylor, Doherty, & Taylor, 2010). Other studies have shown an association between *in utero* exposure to BPA and hypomethylation of specific sites in genes involved in cancer development (Ho, Tang, Belmonte de Frausto, & Prins, 2006).

9. CONCLUSION

The fetus and child are particularly exposed to EDCs. Indeed, several of those EDCs cross the placental barrier or accumulate in maternal milk. Moreover, childhood behavior such as crawling or placing objects in their mouth increases exposure. Because of its high dependence on sex steroids and thyroid hormones for its maturation, the developing organism is very sensitive to any alteration of its hormonal environment.

Epidemiological studies have reported effects of EDCs on major hormone-dependent stages of development: fetal growth, sexual differentiation, puberty, brain development, and energy balance. However, the epidemiological data concerning the EDCs effects on the developing fetus and child are relatively scarce. Besides the challenges listed in Table 1.1, epidemiological studies present several other difficulties. Accurate quantification of exposure throughout pregnancy and childhood is difficult as described for BPA. In addition, for practical reasons, most epidemiological studies have to focus on one group of compounds and ignore other EDCs or risk factors. Genetic susceptibility to endocrine disruption or the effect of associated stresses of other nature is still to be studied.

An analysis of the literature underlines the need for identifying early and fine markers of EDC effects. Animal models can help to unravel the mechanisms of action of EDCs and discover new markers that could underlie alterations of function.

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