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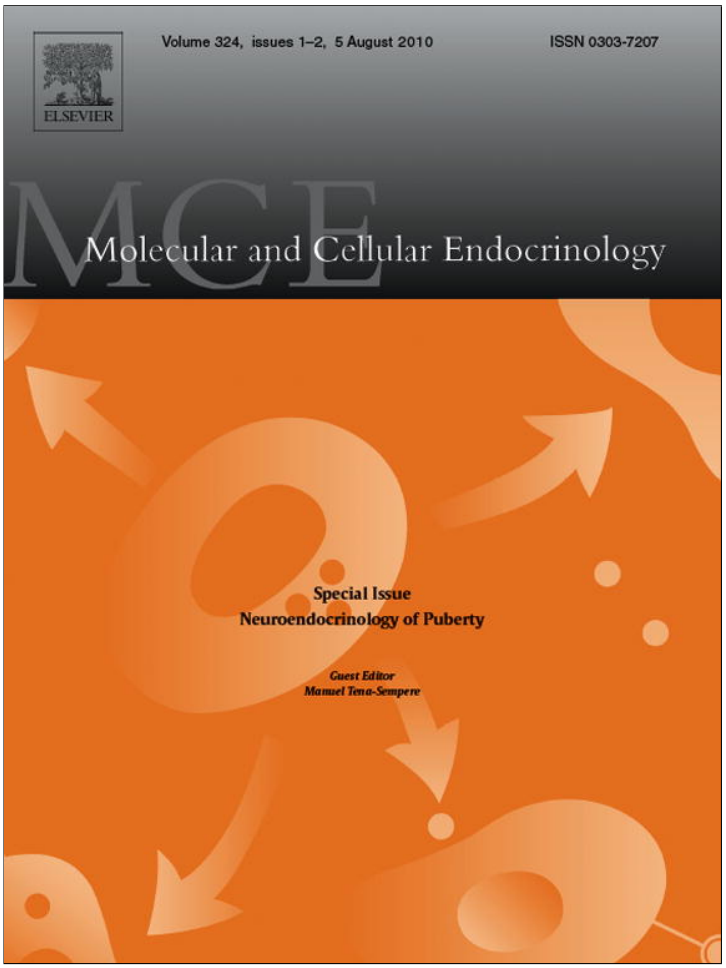

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Review

Neuroendocrine disruption of pubertal timing and interactions between homeostasis of reproduction and energy balance

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ABSTRACT

The involvement of environmental factors such as endocrine disrupting chemicals (EDCs) in the timing of onset of puberty is suggested by recent changes in age at onset of puberty and pattern of distribution that are variable among countries, as well as new forms of sexual precocity after migration. However, the evidence of association between early or late pubertal timing and exposure to EDCs is weak in humans, possibly due to heterogeneity of effects likely involving mixtures and incapacity to assess fetal or neonatal exposure retrospectively. The neuroendocrine system which is crucial for physiological onset of puberty is targeted by EDCs. These compounds also act directly in the gonads and peripheral sex-steroid sensitive tissues. Feedbacks add to the complexity of regulation so that changes in pubertal timing caused by EDCs can involve both central and peripheral mechanisms. In experimental conditions, several neuroendocrine endpoints are affected by EDCs though only few studies including from our laboratory aimed at EDC involvement in the pathophysiology of early sexual maturation. Recent observations support the concept that EDC cause disturbed energy balance and account for the obesity epidemic. Several aspects are linking this system and the reproductive axis: coexisting neuroendocrine and peripheral effects, dependency on fetal/neonatal programming and the many factors cross-linking the two systems, for instance leptin, adiponectin, Agouti Related Peptide (AgRP). This opens perspectives for future research and, hopefully, measures preventing the disturbances of homeostasis caused by EDCs.

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Contents

1. Introduction: rationale for endocrine disruption of pubertal timing 110
2. Sexually dimorphic evidence of endocrine disruption of pubertal timing in humans 111
3. Direct and indirect EDC effects on neuroendocrine maturation at puberty 112
4. Experimental models of EDC neuroendocrine effects on the reproductive axis 113
   4.1. In vitro studies 114
   4.2. In vivo studies and critical periods 114
5. A rodent model of EDC neuroendocrine effects on sexual maturation 115
6. Endocrine disruption of puberty and reproduction in relation to homeostasis of energy balance 116
   6.1. Experimental data 116
   6.2. Human data 118
7. Perspectives 118
   Acknowledgments 118
   References 118

1. Introduction: rationale for endocrine disruption of pubertal timing

Puberty is the life period when pituitary–gonadal maturation leads to a series of physical changes and ultimately, achievement of reproductive capacity. A central event in the onset of puberty is

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an increase in frequency and amplitude of Gonadotropin Releasing Hormone (GnRH) secretion in the hypothalamus. This event is controlled by redundant inhibitory or excitatory mechanisms that respectively disappear or appear at the onset of puberty (Bourguignon, 2004). It is generally agreed that variations in pubertal timing within a physiological 5-year period are predominantly determined by genetic factors while environmental factors play a comparatively minor role (Parent et al., 2003). A robust landmark of environmental effects on pubertal timing arose through the secular advance in menarcheal age. Because this observation was made between the mid-19th and the mid-20th centuries both in USA and Western Europe and more recently in developing countries (Parent et al., 2003), the likely explanation was thought to be improvement in health and nutritional status with industrialization. Accordingly, the end or slowdown of this process seen between 1960 and 2000 was expected. Around the year 2000 however, two large American studies provided evidence of earlier onset of puberty (Herman-Giddens et al., 1997; Lee et al., 2001). Very recently relatively similar findings were obtained in Denmark and Belgium (Aksglaede et al., 2009; Roelants et al., 2009). As opposed to the previous changes, the recent observations were heterogeneous: they could differ among countries; initial signs such as onset of breast development were more affected than subsequent signs such as menarcheal age (Herman-Giddens et al., 1997; Lee et al., 2001; Aksglaede et al., 2009; Roelants et al., 2009); age distribution showed skewing towards earlier ages for initial signs and towards later ages for final signs (Roelants et al., 2009; Papadimitriou et al., 2008). Because those changes in pubertal timing were concomitant with the epidemic of obesity in USA, the pathophysiological involvement of fat mass, possibly through leptin (Herman-Giddens et al., 1997; Lee et al., 2001; Himes, 2006) was hypothesized in that country. However, the recent changes in pubertal timing in Denmark were not associated with changes in adiposity (Aksglaede et al., 2009). Thus other factors including endocrine disrupting chemicals (EDCs) could be involved (Teilmann et al., 2002).

Additional evidence of environmental effects on pubertal timing in humans came from studies in children migrating for international adoption. As cohorts, they appeared to mature earlier than children in the foster countries and in the countries of origin (Proos et al., 1991; Parent et al., 2003). Also, sexual precocity requiring GnRH agonist therapy was much more common in those migrating children than in others (Krstevska-Konstantinova et al., 2001; Teilmann et al., 2006). Based on increased serum levels of DDE, a derivative of the estrogenic insecticide DDT found among migrating children, we hypothesized that early exposure to this EDC and subsequent withdrawal due to migration could account for a neuroendocrine pathogenetic mechanism of secondary central precocious puberty (Krstevska-Konstantinova et al., 2001; Parent et al., 2003). Many other EDCs however could possibly be involved and could result in peripheral precocity as well (see below). The bias accounting for DDT study came from the very long half-life of its derivative DDE. Moreover, it was likely that other factors including recovery from earlier nutritional as well as psychosocial deprivation could play some role in this particular condition (Dominé et al., 2006).

2. Sexually dimorphic evidence of endocrine disruption of pubertal timing in humans

It is challenging to link exposure to particular EDCs and health issues such as disorders of pubertal timing for several reasons (Buck Louis et al., 2008; Diamanti-Kandarakis et al., 2009). Humans as well as animals are likely exposed to a variety of EDCs acting as mixtures with time-related changes in compounds and doses. Mixtures result in more than additive effects since compounds mixed at concentrations that were inactive when used as single EDCs were shown to become active when used as mixtures (Kortenkamp, 2008). The study of mixtures, however, is complex and laborious. So far neuroendocrine studies have been performed with single classes of EDCs only. When exposure is assessed at the time of pubertal disorders, there has been a very long period since fetal/perinatal life, the most critical time for EDC effects. Though such factors could affect the relevance of the studied relationship between EDCs and pubertal disorders, we will review the available information that is mainly based on the study of single EDCs. Prenatal and postnatal exposure will be separated whenever possible as well as effects in males and females. Sexual dimorphism is indeed a critical issue when EDC effects are considered. As a whole, EDCs appear to work either as estrogen agonists or as androgen antagonists with the ratio Estrogen/Androgen actions as an ultimate determinant of EDC effects (Rivas et al., 2002). Consistent with this concept is the observation that premature breast

Table 1
Variations in pubertal timing in relation with pre- and/or post-natal exposure of female humans to endocrine disrupters.

Pubertal timing	Early		Normal		Delayed	
Exposure	Prenatal	Postnatal	Prenatal	Postnatal	Prenatal	Postnatal
DDE (+DDT)	Menarche (Vasiliu et al., 2004)	Menarche (Ouyang et al., 2005)		Menarche (Denham et al., 2005) B2 (Wolff et al., 2008)		
	B2 (Krstevska-Konstantinova et al., 2001)		Menarche and B3 (Gladen et al., 2000)			
Methoxychlor						Monkey (Golub et al., 2003)
PBBs	Menarche (Blanck et al., 2000)		B2 (Blanck et al., 2000)			
PCBs		Menarche (Denham et al., 2005)	Menarche (Vasiliu et al., 2004; Yang et al., 2005)	B2/menarche (Den Hond et al., 2002); B2 (Wolff et al., 2008)		
			Menarche and B3 (Gladen et al., 2000)			
Dioxins			Menarche (Leijs et al., 2008; Warner et al., 2004)	Menarche (Den Hond et al., 2002)	B2 (Leijs et al., 2008)	B2 (Den Hond et al., 2002)
Phthalates		B2 (Colon et al., 2000)				
Phytoestrogens soy formula				Menarche (Strom et al., 2001)		B2 (Wolff et al., 2008)

DDE: dichlorodiphenyldichloroethene; DDT: dichlorodiphenyltrichloroethane; PBB: polybrominated biphenyl; PCB: polychlorinated biphenyl; and B2, B3: Tanner's stages 2 and 3 of breast development.

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Table 2
Variations in pubertal timing in relation with pre- and/or post-natal exposure of male humans to endocrine disrupters.

Pubertal timing	Early		Normal		Delayed	
Exposure	Prenatal	Postnatal	Prenatal	Postnatal	Prenatal	Postnatal
DDE (+DDT)			G3-5, PHV (Gladen et al., 2000)			
PCBs			G3-5, PHV (Gladen et al., 2000) G, TV (Mol et al., 2002)		Penile length (Guo et al., 2004)	P and G (Den Hond et al., 2002)
Dioxin				P and G (Den Hond et al., 2002)		

DDE: dichlorodiphenyldichloroethene; DDT: dichlorodiphenyltrichloroethane; PCB: polychlorinated biphenyl; G: Tanner's stage of genital development; P: Tanner's stage of pubic hair development; PHV: peak height velocity; and TV: testicular volume.

development can occur after exposure to phthalates that are considered to act primarily as androgen antagonists (Colon et al., 2000). Further, an estrogenic compound such as DDT can generate the anti-androgenic sub-product DDE that can also indirectly reflect previous exposure to DDT, further complicating the elucidation of estrogenic versus anti-androgenic effects (Rasier et al., 2008).

Virtually, any clinical evidence of pubertal development (except testicular growth) can result from either centrally driven maturation involving the hypothalamic–pituitary system or direct peripheral interaction in the tissues targeted by sex steroids or both mechanisms. Here, the clinical manifestations will be reviewed irrespective of the underlying mechanism. In a subsequent section, the neuroendocrine mechanisms will be delineated. More data were obtained in girls (Table 1) than in boys (Table 2). This could involve methodological biases since a precise timer of maturation is provided by menarcheal age in girls who also experience more obvious onset of puberty with the development of breasts as opposed to the less perceptible increase in testicular volume in boys (Parent et al., 2003).

While the majority of EDCs studied in girls accounted for normal or early timing (Table 1), dioxins and phytoestrogens were associated with delayed timing of breast development (Den Hond et al., 2002; Leijs et al., 2008; Wolff et al., 2008). Menarcheal age however did not seem to be affected by dioxins or phytoestrogens in the same studies (Den Hond et al., 2002; Leijs et al., 2008) and in others (Strom et al., 2001; Warner et al., 2004). Likewise, dissociation between normal timing of breast development and early menarche was reported in relation to exposure to PBBs (Blanck et al., 2000). These findings emphasize the importance of studying different pubertal signs possibly involving different mechanisms at several times throughout the pubertal process. Except one study reporting early menarche in relation to exposure to PCBs (Denham et al., 2005), this EDC was found to be associated with normal timing of both breast development and menarche (Gladen et al., 2000; Den Hond et al., 2002; Vasiliu et al., 2004; Yang et al., 2005; Wolff et al., 2008). Early breast development (Krstevska-Konstantinova et al., 2001) and early menarche (Vasiliu et al., 2004; Ouyang et al., 2005) were reported in relation to exposure to DDT and or DDE whereas normal pubertal timing was found by others (Gladen et al., 2000; Denham et al., 2005; Wolff et al., 2008). In the female monkey, delayed nipple growth and short follicular phase were seen after exposure to methoxychlor (Golub et al., 2003). Overall, those studies did not enable to show different effects depending on prenatal or postnatal period of exposure to the EDCs.

In boys, the few studies available (Table 2) suggest no effects of DDT, DDE and dioxins (Gladen et al., 2000; Den Hond et al., 2002). Exposure to PCBs was found to be associated with either normal (Gladen et al., 2000; Mol et al., 2002) or delayed timing (Den Hond et al., 2002; Guo et al., 2004) of male pubertal development.

3. Direct and indirect EDC effects on neuroendocrine maturation at puberty

Puberty involves a cascade of physiological events resulting from CNS and neuroendocrine maturation (Table 3). Because sex steroids can act at the different levels of the hypothalamic–pituitary–gonadal system, the imbalance between estrogen and androgen effects caused by EDCs (Rivas et al., 2002) potentially affects all those levels. Gender dimorphism is another important aspect both peripherally and centrally.

Changes in gonads and peripheral tissues responsive to sex steroids were the first endpoints studied to show EDC effects. Direct disruption of the peripheral reproductive system involves predominantly effects mimicking estrogens in the female while counteracted androgen effects are involved peripherally in the male (Diamanti-Kandarakis et al., 2009). Since the reproductive system is regulated by feedback loops between gonads and peripheral tissues on the one hand and pituitary gland, hypothalamus and CNS on the other hand, direct peripheral effects of EDCs can secondar-

Table 3
Some possible mechanisms of EDC maturational effects on the hypothalamic–pituitary–gonadal system in female and male individuals.

Level possibly targeted by EDCs	Developmental effects of increased Estrogen/Androgen balance		Mechanisms
	Female	Male	
CNS: suprahypothalamic afferences	Structural changes?		Primary central/neuroendocrine or Secondary to altered feedback effects of gonadal hormones
Hypothalamus: GnRH neurons and surrounding neurono-glial system	Facilitation (or inhibition) of pulsatile GnRH secretion Female more sensitive than the male? Alteration of sexually dimorphic control of ovulation		
Pituitary gland: gonadotrophic cells	Early pubertal stimulation or Increased prepubertal inhibition (negative feedback)		Response to neuroendocr. effects or Peripheral feedback
Gonads: sex steroid production/effects and gametogenesis	Alteration of folliculogenesis	Alteration of spermatogenesis	Primary peripheral or Secondary to altered neuroendocrine control
Peripheral tissues: sex steroid effects	Early/increased stimulation of estrogen sensitive tissues (breast, uterus)	Delayed/reduced stimulation of androgen sensitive tissues (penis, prostate)	

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Table 4
In vitro evidence of neuroendocrine disruption of the reproductive system.

EDC	Expos.	Sex	Endpoint
PCBs	GT1–7 neurons	–	Aroclor1221: increased GnRH mRNAs and peptide release; neurite outgrowth. Aroclor 1254: biphasic effects and neurotoxicity (Gore et al., 2002)
	GT1–7 neurons	–	Early elevation and late reduction of GnRH release; apoptotic and neurotoxic effects (Dickerson et al., 2009)
MXC	GT1–7 neurons	–	ER independent reduction of GnRH mRNAs; ER mediated stimulation of GnRH release (Gore, 2002)
Coumestrol	GT1–7 neurons	–	ERβ mediated inhibition of GnRH mRNA expression (Bowe et al., 2003)
DDT	Rat hyp. explants	F	ER and AhR receptor mediated stimulation of pulse frequency of GnRH secretion and glutamate-evoked release (Rasier et al., 2007, 2008)
Dioxin	Adult rat	F	TCDD in vitro: no effect on pulsatile GnRH release (Trewin et al., 2007)

MXC: methoxychlor; PCBs: polychlorinated biphenyls; ER: estrogen receptor; AhR: arylhydrocarbon receptor; DDT: dichlorodiphenyltrichloroethane; and TCDD: 2,3,7,8-tetrachlorodibenzo-p-dioxin.

ily influence central mechanisms through changes in endogenous peripheral hormones. Consequently, changes in neuroendocrine and pituitary function could result indirectly from altered peripheral feedback.

Changes in hypothalamic and pituitary function could also result directly from EDC neuroendocrine effects. Centrally, the neuroendocrine control of reproduction through the preovulatory gonadotropin surge and its alteration following exposure to sex steroids during fetal or perinatal life has been known for several decades as a specific female feature (Gorski, 1968). In such conditions, changes in gonadal and peripheral tissue function could be determined by EDC neuroendocrine effects. Due to obvious limits in assessment of neuroendocrine function in the clinical setting, the use of experimental models is required to tackle neuroendocrine effects of EDC.

4. Experimental models of EDC neuroendocrine effects on the reproductive axis

In Tables 4 and 5 are summarized some findings providing experimental evidence of EDC neuroendocrine effects. The available data are quite heterogeneous as far as the studied EDCs, the experimental models and the endpoints. Since, so far, only few studies have addressed the issue of neuroendocrine disruption of sexual maturation, we have extended our review of the mecha-

Table 5
In vivo evidence of neuroendocrine disruption of the reproductive system.

EDC	Expos.	Sex	Endpoint
DES	Quail embryo	M	Reduced vasotocin in medial preoptic nucleus and bed nucleus of stria terminalis; suppression of male copulatory behavior (Viglietti-Panzica et al., 2005)
MXC	Fetal ewe	F	Delayed LH surge (Savabieasfahani et al., 2006) – secondary to altered folliculogenesis?
BPA	Fetal ewe	F	Reduced magnitude of LH surge (Savabieasfahani et al., 2006) – secondary to altered folliculogenesis?
	Fetal rat	M	Increased ERβ mRNA expression in POA (Ramos et al., 2003)
	Fetal/neonatal mouse	F	Decline in tyrosine hydroxylase neurons in POA and suppression of sex-related rearing behavior (Rubin et al., 2006)
	Neonatal rat	M	Increased anxiety behavior and body weight (Patisaul and Bateman, 2008)
	Neonatal rat	M/F	Reduced hypothalamic Kiss-1 expression (Navarro et al., 2009)
	Adult OVX rat	F	Increased progesterone receptor expression in POA and VMN; reduced sexual receptivity (Funabashi et al., 2003)
DDT	Neonat. mouse	M	Increased brain estrogen receptor (Mussi et al., 2005)
	Neonat. fish	M/F	Increased activity and expression of brain aromatase and male to female sex reversal (Kuhl et al., 2005)
	Neonat. rat	F	Increased frequency of pulsatile GnRH secretion, sexual precocity, disturbed estrus cyclicity (Rasier et al., 2007)
PCBs	Fetal rat	F	Aroclor 1221: increased GnRH mRNAs in POA-anterior hypothalamus and unchanged timing of vaginal opening; Aroclor 1254: no effects (Gore, 2008)
	Fetal rat	F	Aroclor 1221: altered female mating behavior (Steinberg et al., 2007) and reduced proestrus LH surge (Steinberg et al., 2008)
	Adult fish	M	Aroclor 1254: reduced tryptophan hydroxylase activity and GnRH content in POA (Khan and Thomas, 2001)
Dioxin	Fish larvae		TCDD: prevention of brain aromatase upregulation by estradiol (Cheshenko et al., 2007)
	Fetal rat	M	TCDD: increased content and reduced release of GnRH by hypothalamic explants (Clements et al., 2009)
Isoflavone	Adult rat	F	Increased ERβ receptors in PVN; reduced upregulation of oxytocin receptors via ERα in VMN; reduced sexual receptivity (Patisaul et al., 2001)
Coumestrol	Adult OVX rat	F	Reduced multiunit electrical activity in hypothalamus and inhibition of LH pulse amplitude and frequency (McGarvey et al., 2001)

MXC: methoxychlor; BPA: bisphenol A; DES: diethylstilbestrol; PCBs: polychlorinated biphenyls; PVN: paraventricular nucleus; VMN: ventromedial nucleus; POA: preoptic area; OVX: ovariectomized; ER: estrogen receptor; DDT: dichlorodiphenyltrichloroethane; and TCDD: 2,3,7,8-tetrachlorodibenzo-p-dioxin.

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nisms of EDC effects to the overall neuroendocrine control of the reproductive system.

4.1. In vitro studies

In vitro models allowed direct evaluation of EDC effects on neurono-glial function (Table 4). A debated question is as to whether sex steroids and EDCs that have the estrogen receptor as common target influence the GnRH neuron directly or indirectly. In a previous review, we concluded that GnRH neurons could be directly involved in responding to estrogens (Matagne et al., 2003). Maturational characteristics could influence estrogen effects on GnRH neurons. GT1 cells, a model for GnRH neurons that are responsive to estrogens and EDCs, have been obtained from immortalization at an embryonic stage and are somehow developmentally arrested. Direct early responsiveness of GnRH neurons to estrogens at early developmental stage is supported by a recent study showing that neurogenesis in cultured olfactory placode was directly stimulated by estradiol (Agça et al., 2008). Later in adulthood, estrogen effects on GnRH neurons may become indirect since studies with cell specific deletion of the different ER subtypes indicated that estradiol positive feedback at the time of the preovulatory surge primarily involved other estrogen responsive cells than GnRH neurons (Herbison, 2008). Species differences could also occur since it was shown recently that orchidectomy caused increased amplitude of pulsatile GnRH release from monkey but not from rat hypothalamic explants (Woller et al., 2010). Using cultured immortalized GnRH neurons, variable changes in GnRH transcripts and peptide release were obtained depending on the EDC and the concentrations used, the response being possibly non-linear and biphasic (Gore et al., 2002; Gore, 2002; Bowe et al., 2003; Dickerson et al., 2009). PCBs also accounted for neurotoxic effects with increased expression of cleaved caspase-9 (Gore et al., 2002; Dickerson et al., 2009). We used hypothalamic explants to study the effects of DDT, an estrogenic EDC, in relation to sexual maturation. Our paradigm included axons and terminals of the final effector, i.e. the GnRH neuron (Purnelle et al., 1997) as well as the afferent neurono-glial apparatus possibly involved in EDC effects. The explants also retained some developmental characteristics since they released GnRH in a pulsatile manner with a frequency increasing from birth to onset of puberty (Bourguignon et al., 1992). We used hypothalamic explants of immature female rats aged 15 days because they were found earlier to be responsive to estradiol through an increase in frequency of pulsatile GnRH secretion (Matagne et al., 2004). In such conditions, DDT resulted in effects similar to estradiol (Rasier et al., 2008). However, Trewin et al. (2007) could not observe any change in pulsatile GnRH secretion caused by dioxin using hypothalamic explants from cycling adult female rats. The age difference could account for such discrepant observations since we did not observe at 25 and 50 days the stimulatory estradiol effects seen at 5 and 15 days (Matagne et al., 2004). Though such in vitro models provided an opportunity to study directly the mechanisms of neuroendocrine EDC effects, the conditions were not comparable to those in vivo both in terms of GnRH neuron function/regulation and environmental exposure to the studied EDCs. The explants or the immortalized neurons were deafferented from physiological neurono-glial inputs. Concentrations higher than those toxicologically relevant in humans were required likely due to low diffusion in the explants (Matagne et al., 2003). This reinforced the value of in vivo models.

4.2. In vivo studies and critical periods

Suggestive evidence of EDC neuroendocrine effects was obtained in vivo by studying physiological processes known to involve hypothalamic or CNS regulation (Table 5). Except phytoestrogen effects that were studied in adult animals, the vast majority of studies were performed after exposure during fetal and/or early postnatal life. This is consistent with the concept of critical periods in early life that appeared to determine the effects of sex steroids (McCarthy et al., 2009) as well as nutrition (Gluckman and Hanson, 2004) on the homeostasis of reproduction and energy balance. The neuroendocrine events affected by EDCs in vivo include central, i.e. gonadotropin-dependent onset of puberty (Rasier et al., 2007; Gore, 2008), ovulation that is dependent on stimulation by the gonadotropin surge (Savabieasfahani et al., 2006; Steinberg et al., 2008) and sexual behavior in males (Viglietti-Panzica et al., 2005) and females (Patisaul et al., 2001; Funabashi et al., 2003; Rubin et al., 2006; Steinberg et al., 2007). Not unexpectedly, those three sexually dimorphic processes are likely regulated by sex steroids and possibly disrupted by EDCs differently in males and females in different species. Prenatal exposure of the ovine fetus to testosterone caused alteration of pubertal timing and estrus cyclicity through neuroendocrine alteration of estradiol positive feedback (Unsworth et al., 2005), including marked reduction of FOS-positive GnRH neurons in response to estradiol (Wood et al., 1996). In similar conditions, fetal lamb exposure to the EDCs methoxychlor or BPA accounted for delayed or severely reduced LH surge, respectively, without change in pubertal timing (Savabieasfahani et al., 2006). As emphasized by the authors, the exposed animals also had ovarian anomalies, growth retardation and metabolic disorders that could contribute to disrupted reproductive function. A single prenatal administration of PCB mixture on gestational day 16 caused postnatal growth retardation (Gore, 2008) and disturbed sexual behavior, prominently in females (Wang et al., 2002; Steinberg et al., 2008). Behavioral evidence of transgenerational effects came from female preference of male with no history of exposure, three generations after the progenitors were exposed to vinclozolin (Crews et al., 2007). These findings might suggest epigenetic changes in the neuroendocrine components of sexual behavior regulation. Direct evidence of epigenetic mechanisms in the hypothalamus in relation with sexual differentiation further provide rationale for studies on EDC effects on epigenetics of the neuroendocrine system (Murray et al., 2009).

Other endpoints in experimental studies on neuroendocrine effects include expression or transcripts of sex steroid receptors (Patisaul et al., 2001; Funabashi et al., 2003; Ramos et al., 2003; Mussi et al., 2005) and enzymes involved in sex steroid metabolism or dependent on sex steroid effects (Khan and Thomas, 2001; Kuhl et al., 2005; Rubin et al., 2006). In this respect, aromatase deserves special attention due to its involvement in the control of sexually differentiated neuroendocrine functions including sexual behavior (Balthazart et al., 2006). In the zebra fish, dioxins were shown to alter estrogen upregulation of aromatase (Cheshenko et al., 2007). Several authors including our group have studied direct or indirect appraisal of GnRH synthesis and secretion (Khan and Thomas, 2001; McGarvey et al., 2001; Rasier et al., 2007; Gore, 2008) While we found that in vivo early postnatal exposure of female rat to DDT, an estrogenic EDC, resulted in premature developmental increase in GnRH pulse frequency in vitro (Rasier et al., 2007), Clements et al. (2009) reported recently that, in male rats, GnRH release in vitro was reduced after fetal exposure to dioxin, another estrogenic EDC. This stresses again the diversity of conditions including gender, compound and period of exposure that could account for discrepant data. Finally, neuropeptides dependent on sex steroid effects (Patisaul et al., 2001; Viglietti-Panzica et al., 2005) including the recent demonstration of reduced Kiss-1 expression by BPA (Navarro et al., 2009) and non-sexual aspects of behavior (Patisaul and Bateman, 2008). In summary, several neuroendocrine studies on EDC effects were performed but the conclusions drawn from such studies remain rather limited due to the many variables that may influence the response to EDCs, e.g. dose, age at exposure,

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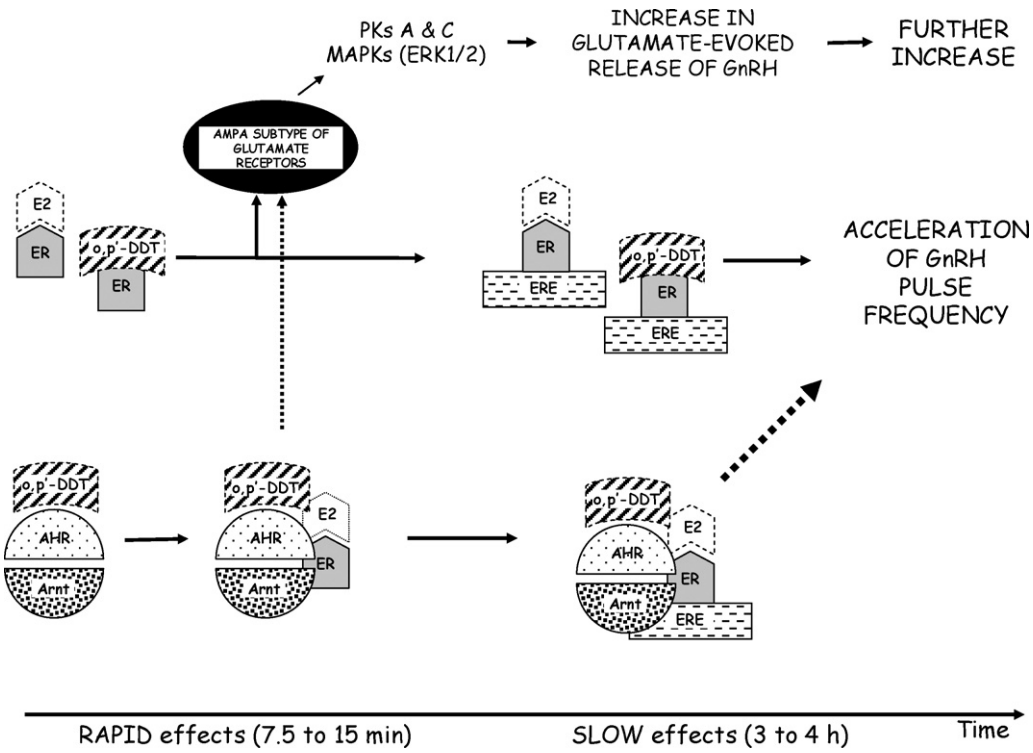

Fig. 1. Schematic illustration of the mechanisms of estradiol and o,p′-DDT effects on GnRH secretion. Three pathways are identified with both rapid, presumably non-genomic effects and slow, presumably genomic effects: (1) binding the classical estrogen receptor (ER) and causing genomic effects after activation through the estrogen responsive element (ERE); (2) binding some ER and interacting with the AMPA subtype of glutamate receptor with subsequent involvement of intracellular kinases; and (3) binding the aryl hydrocarbon (AHR) orphan dioxin receptor, and cross talking with the E2 signalling machinery to form a functional transcriptional complex in the regulatory region of ER-responsive genes (Ohtake et al., 2003).

duration of exposure, route of administration, gender and mixtures. Comparison between neuroendocrine effects and studies on effects in other parts of the CNS may help in elucidation of alterations caused by EDCs during CNS development (Naveau et al., 2010).

5. A rodent model of EDC neuroendocrine effects on sexual maturation

Using hypothalamic explants of immature female rats aged 15 days, we showed that o,p′-DDT directly stimulated GnRH pulse frequency in vitro. The mechanism (Fig. 1) involved both the ER and the aryl hydrocarbon receptor (AHR) as well as the AMPA subtype of glutamate receptors (Rasier et al., 2008). The DDT effects were not only dose-dependent but also time-dependent with both rapid and slow effects since the glutamate-evoked release of GnRH increased as soon as after 7.5 min of incubation and further after 4 h of incubation (Rasier et al., 2008). These in vitro findings provided the rationale for an in vivo study of DDT effects on pubertal timing through a central/hypothalamic mechanism. The aim was to expose female rats early and transiently to DDT in order to model the neuroendocrine effects of the pesticide that could account for sexual precocity in girls migrating for international adoption (Krstevska-Konstantinova et al., 2001; Parent et al., 2003). Because fetal or early postnatal exposure to testosterone or estradiol would masculinize the CNS and alter the mechanism of estrus cycling, the animals were exposed to DDT on postnatal days 6–10. We reported in earlier studies that exposure to estradiol within this age window resulted in sexually differentiated effects on GnRH secretion both in vitro and in vivo and subsequent female sexual precocity (Matagne et al., 2004). In similar age conditions, in vivo exposure to DDT followed by ex vivo study of GnRH release by hypothalamic explants resulted in premature acceleration of pulsatile GnRH secretion (Rasier et al., 2007). Both vaginal opening and first estrus occurred earlier after exposure to estradiol or DDT though the time interval between the two events was increased. Our findings could involve central as well as peripheral mechanisms of sexual precocity. In order to obtain further evidence of neuroendocrine effects of DDT in vivo, the response of pituitary LH to a bolus administration of synthetic GnRH was studied. LH response was shown to reflect previous stimulation by endogenous GnRH and, in man, increased LH response was regarded as the evidence of neuroendocrine maturation leading to the so-called central puberty (Carel et al., 2009). A different situation occurred in the female rat since neuroendocrine maturation was associated with a developmental reduction of LH response, a confounding observation with LH reduction due to negative feedback such as seen in peripheral precocity. Nevertheless, we observed a premature developmental reduction in LH response after exposure to DDT that possibly resulted from neuroendocrine effects (Rasier et al., 2007). Based on the rodent model, our interpretation of sexual precocity after migration is summarized in Fig. 2 (Parent et al., 2003; Rasier et al., 2006, 2007). During exposure to DDT, estrogenic effects can account for both peripheral and central (neuroendocrine) stimulation. However, due to concomitant negative feedback inhibition at the pituitary level, the central effects are not translated into gonadotropin stimulation of the ovaries until the pituitary inhibition disappears following migration in a DDT-free environment. In a study of internationally adopted girls aged 5–8 years before they eventually showed clinical evidence of sexual precocity, Teilmann et al. (2007) reported that serum FSH and estradiol levels were already elevated in several girls, confirming early pituitary–ovarian activity after migration. The above mechanism is comparable to that operating in other conditions with peripheral precocious puberty (e.g. congenital adrenal hyperplasia, adrenal or gonadal tumours) followed by secondary central precocious puberty after the peripheral disorders is cured by medical or surgical treatment (Parent et al., 2003). Consistent with this

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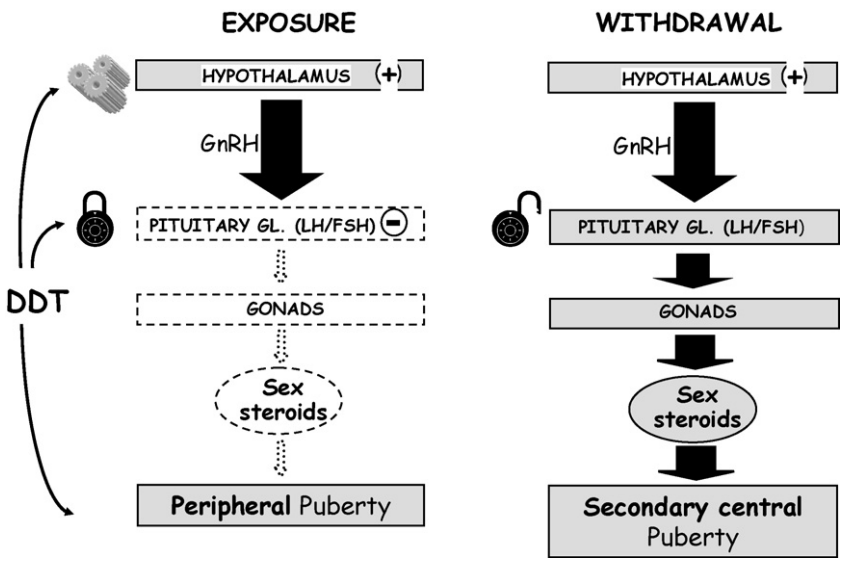

Fig. 2. Schematic representation of the putative mechanism of sexual precocity after transient exposure to the estrogenic insecticide DDT in girls migrating for international adoption. During exposure to DDT, neuroendocrine maturation is promoted but not translated into ovarian stimulation due to negative feedback inhibition of the pituitary gonadotropes. After migration in a DDT-free environment, the negative feedback does no longer operate and allows central maturation to lead to gonadotropin-dependent puberty (modified from Rasier et al., 2006).

concept, central precocity should not be manifested in conditions of persisting exposure to DDT. We attempted to demonstrate that continued administration of DDT to the female rats was not associated with evidence of precocious central maturation but we failed due to toxic effects including malnourishment and growth failure (Rasier et al., 2007).

We previously reviewed the effects of EDCs on sexual maturation in laboratory rodents (Rasier et al., 2006). Peripheral effects can be biphasic, low doses causing early puberty and high doses delayed puberty such as shown using triphenyltin in the female rat (Grote et al., 2006) and phthalates in the male (Ge et al., 2007). In the few studies where hypothalamic pituitary function was assessed, LH secretion was reduced together with early vaginal opening after DES (Kubo et al., 2003) or irregular cycling after BPA (Rubin et al., 2006). Still, the possibly coexisting central and peripheral effects remain a matter of confusion. As an example, BPA administration to neonatal female rats for 4 days resulted in early vaginal opening and acyclicity but no change in sexual receptivity and FOS induction in GnRH neurons after steroid priming (Adewale et al., 2009). This suggested predominant peripheral effects in those conditions and highlighted the importance of simultaneous study of central and peripheral effects.

6. Endocrine disruption of puberty and reproduction in relation to homeostasis of energy balance

The neuroendocrine system in the hypothalamus constitutes a single place for different aspects of homeostasis including reproduction and energy balance. It is also involved, during fetal and neonatal life, in programming mechanisms accounting for the "developmental origin of health and diseases" (Gluckman and Hanson, 2004). Therefore, it appeared interesting to integrate some of the many connections between homeostasis of reproduction and homeostasis of energy balance, during both prenatal/neonatal and postnatal life (Fig. 3).

6.1. Experimental data

Fetal malnourishment as well as fetal exposure to endocrine disrupters such as DES and BPA were shown to possibly result in low birth weight, early puberty, ovulatory disorders, obesity in adulthood and metabolic syndrome (Gluckman and Hanson, 2004; Newbold et al., 2008, 2009; Sloboda et al., 2009; Heindel and vom Saal, 2009). The consequences of fetal malnourishment on adult adiposity excess and metabolic syndrome could be prevented by neonatal or early postnatal leptin treatment indicating the critical role of this peripheral peptide in fetal/neonatal programming (Vickers et al., 2005). Leptin has appeared to be not only an anorexigenic hormone produced by the mature adipocyte but also a structural organizer of the hypothalamic circuitry controlling energy balance during a critical period including fetal life and the first 3 weeks of postnatal life in rodents (Bouret et al., 2004; Bouret and Simerly, 2007). Bouret (2010) hypothesized that anomalies in early leptin organizational effects in relation with nutritional disturbances during that critical window could predispose to later obesity and metabolic disorders. Leptin could also be involved after fetal/neonatal exposure to sex steroids since neonatal androgenization resulted in marked reduction of leptin mRNA levels in the pituitary gland of female rats on days 14 and 22 (Morash et al., 2001). Further involvement of leptin in relation to sex steroids and EDCs was suggested by the reduced serum leptin levels observed neonatally in association with reduced anogenital distance after fetal exposure to phthalates (Boberg et al., 2008). During postnatal life, leptin is also obviously an important link between energy balance and reproduction. As shown in Fig. 4, facilitatory effects on GnRH secretion were observed after a single leptin administration in 15-day-old rats but not at 50 days, further supporting the concept of early critical window before the age of 3 weeks in rodents (Parent et al., 2003; Lebrethon et al., 2007). Together with leptin as peripheral messenger, a common hypothalamic mediator of early EDC effects on energy balance and reproduction could be the Agouti Related Peptide (AgRP), an endogenous orexigenic antagonist at melanocortin receptors. In our laboratory, AgRP treatment in vivo or in vitro (Fig. 4) was found to cause deceleration of frequency of pulsatile GnRH secretion from male rat hypothalamic explants (Lebrethon et al., 2007). This effect was similarly seen in immature and pubertal animals suggesting a role in the neuroendocrine control of the reproductive axis but no involvement in the mechanism of puberty. Exposure of fetal and neonatal mice to BPA was shown to result in hypomethylation of a metastable epiallele locus upstream

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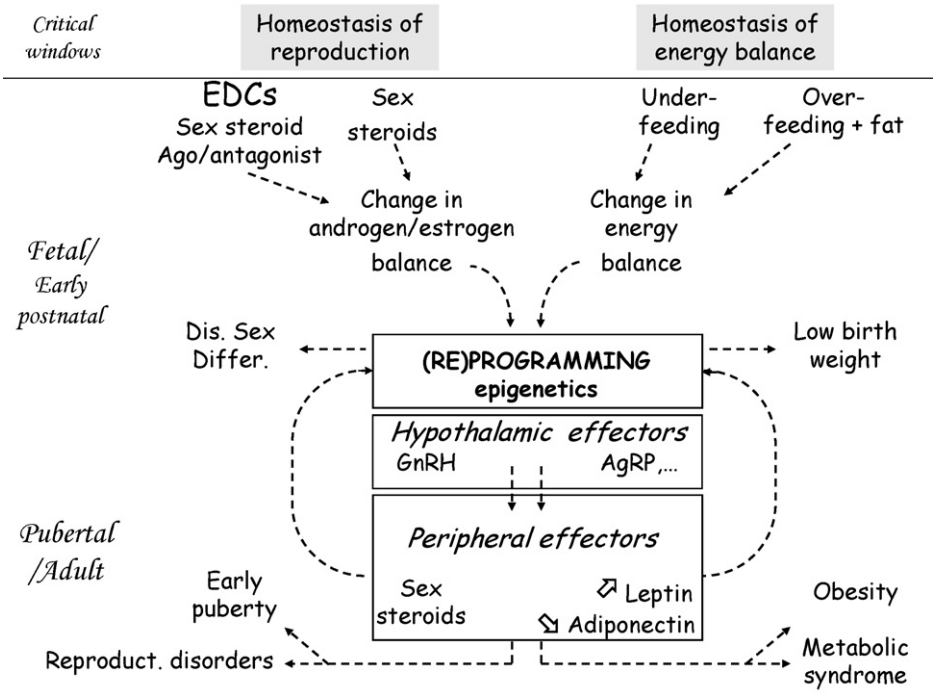

Fig. 3. Schematic representation of the interactions between homeostasis of reproduction and energy balance as well as hypothalamic effectors and peripheral effectors in the neuroendocrine mechanisms involved in effects on pubertal timing.

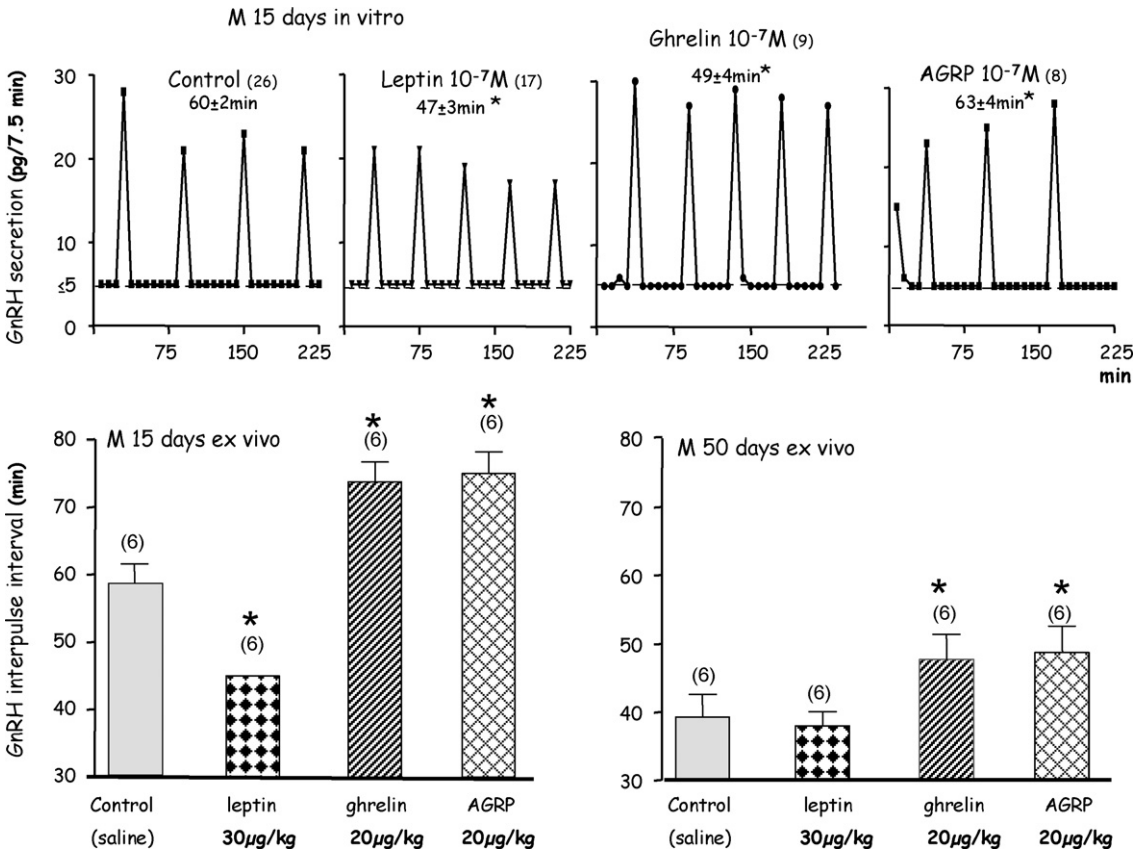

Fig. 4. Upper panel: representative profiles of GnRH secretion by hypothalamic explants from 15-day-old male rats incubated in the presence of leptin, ghrelin or Agouti-related peptide (AgRP). In each condition, the mean ± SD interpulse interval is given (number of explants studied). Lower panel: mean interpulse interval observed in vitro using explants from 15- or 50-day-old male rats injected with leptin, ghrelin or AgRP in vivo. $^{*}p < 0.05$ versus controls (modified from Lebrethon et al., 2007).

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to the agouti gene that was associated with increased ectopic agouti gene expression (Dolinoy et al., 2007) known to result in yellow coat color and adult obesity (Dolinoy, 2008). Fertility however was not impaired in those BPA exposure conditions and adult weight was not studied.

EDCs affect adipocytes directly at different periods of life through induction of cell differentiation and adipogenesis (Grün et al., 2006) as well as lipid accumulation in differentiated adipocytes (Wada et al., 2007). Gestational and lactational exposure to BPA resulted in increased adipogenesis at weaning in female rats (Somm et al., 2009). This is in contrast to the reduced adiposity and increased insulin sensitivity in mice fed from conception to adulthood with a phytoestrogen rich diet (Cederroth et al., 2008). EDC effects on adipocytes could be increased due to storage in the adipose tissue of several EDCs that are lipophilic. Adiponectin, a factor protecting against obesity and insulin resistance, could play a role as well. Using mature abdominal adipocytes in vitro, BPA reduced the secretion of several adipokines including adiponectin (Hugo et al., 2008; Ben-Jonathan et al., 2009). Through such an effect, EDC could facilitate GnRH secretion since immortalized GnRH neurons were shown to express adiponectin receptors and GnRH release was reduced in the presence of adiponectin (Wen et al., 2008). The fetal balance between estrogenic and androgenic steroids that is disturbed by EDCs can also be directly affected by sex steroids such as treatment of pregnant ewe with testosterone. In these conditions, pubertal timing in female lambs was shifted from the female to the male pattern as a function of the degree of virilization of external genitalia (Kosut et al., 1997). Postnatal events may worsen the consequences of disturbed programming: postnatal weight excess due to overfeeding amplified the reproductive disturbances following prenatal androgenization of the female lamb (Steckler et al., 2009).

6.2. Human data

In the clinical setting, the interaction between nutrition and reproduction has been viewed for almost 40 years as an issue for adolescent and adult females in the perspective of energy availability determining reproductive system function. Such a concept that was proposed by Frisch and Revelle (1970) became further substantiated when leptin was discovered as a key messenger from adipose tissue to the hypothalamus and controlling the reproductive system via stimulation of GnRH secretion (rev in Parent et al., 2003). As already stated in the introduction, such a mechanism substantiated a putative link between obesity epidemics and earlier onset of puberty. In the field of endocrine disruption, fetal and neonatal determination of reproductive disorders in adulthood arose four decades ago following the observations of genital cancer in the female offspring of mothers treated with DES during pregnancy (Herbst et al., 1971). Similarly consistent with the early life origins of diseases, it was suggested recently that obesity and metabolic syndrome could involve primary peripheral mechanisms through early impact of nutrition on increased adipogenic and lipogenic capacity of adipocytes (Muhlhausler and Smith, 2009). Such a concept is consistent with the recent finding that fast weight gain in infancy between birth and 9 months predicts increased adiposity at 10 years and early menarcheal age (Ong et al., 2009). The hypothesis was also raised that EDC effects on adipose tissue and energy balance could provide the basis of a toxicological mechanism in the obesity epidemic (Baillie-Hamilton, 2002; Heindel, 2003). All together, those findings indicate that both the human reproductive system and energy balance system share a possible fetal/early postnatal determinism of adult disorders under the influence of early nutritional conditions and/or early exposure to EDCs.

Ibáñez et al. (1998, 2007) reported that intrauterine growth retardation (IUGR) was associated with increased risk of premature pubarche, hyperinsulinism, ovarian hyperandrogenism and polycystic ovary syndrome (PCOS). These findings can be correlated with several experimental effects of manipulation of nutrition or exposure to sex steroids or EDCs in the fetus as detailed above. Of particular interest is the context of this discussion is the observation that IUGR is associated with increased visceral adiposity and reduced serum levels of adiponectin in childhood (Ibáñez et al., 2009). The latter effect parallels BPA-induced reduction of adiponectin production by adipocytes (Hugo et al., 2008; Ben-Jonathan et al., 2009). In addition, breast-fed IUGR newborns show lower adiposity and higher serum leptin levels than controls (Ibáñez et al., 2010), an interesting finding in the face of prevention of insulin resistance and obesity by postnatal leptin administration in the rat born IUGR (Vickers et al., 2005).

7. Perspectives

Understandably, the main focus of studies on EDCs has been the reproductive system for several decades with fertility and hormone-dependent cancers as the most critical issues. More recently, genital malformations and disorders of pubertal timing have emerged as earlier manifestations of a spectrum of disturbances all likely to result from EDC effects during the fetal and neonatal critical age windows. While the interactions between nutrition and reproduction have been known for decades, it is only recently that EDCs have appeared to alter the homeostasis of both reproduction and energy balance. These two systems share neuroendocrine control in relation with feedback from peripheral tissues through circulating factors. Further studies should scrutinize the common mechanisms and factors possibly linking EDCs with disturbances of those two systems.

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