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G-Protein Coupled Hormone Receptors of the Hypothalamic-Pituitary-Gonadal Axis are Targets of Endocrine Disrupting Chemicals

Valentine Suteau, Patrice Rodien and Mathilde Munier

Abstract

Endocrine-disrupting chemicals have received significant concern, since they ubiquitously persist in the environment and are able to induce adverse effects on health, and more particularly on reproductive function. Most of the studies focused on nuclear hormone receptors as mediators of sex steroid hormones signaling. However, there are increasing evidences that peptides hormones of the Hypothalamo-Pituitary-Gonadal axis are targets of endocrine-disrupting chemicals (as Gonadotropin-Releasing Hormone, Follicle-Stimulating Hormone, Luteinizing Hormone…). The majority of these hormones act on G protein-coupled membrane receptors. This review summarizes the effects of endocrine-disrupting chemicals on homeostasis of peptides hormone of Hypothalamo-Pituitary-Gonadal axis and on their G protein-coupled membrane receptors signaling revealed by experimental, clinical, and epidemiological studies in human.

Keywords: G-protein coupled hormone receptors, hypothalamic-pituitary-gonadal axis, hormones, endocrine-disrupting chemicals

1. Introduction

Public concern of endocrine-disrupting chemicals (EDCs) has been rising since the 1990s. EDCs are defined as "an exogenous substance or mixture that alters function(s) of the endocrine system and consequently causes adverse health effects in an intact organism, or its progeny, or (sub)populations" [1–3]. EDCs are found in many products comprising plasticizers, personal care products, pesticides… [1]. Humans are constantly exposed to several different EDCs by ingestion, inhalation, and dermal contact. Some classes of EDCs have been studied in detail. Here, we selected three classes of EDCs based on knowledge of their effects on Hypothalamo-Pituitary-Gonadal (HPG) axis: bisphenol A (BPA), phthalates and dichlorodiphenyltrichloroethane (DDT). BPA is one of the most massively produced EDC with over three million tons manufactured annually [4]. It is used in food packaging, toys, resins used in canned, and medical equipment. Because its incomplete

polymerization and its release from polycarbonate at high temperature, exposure to BPA is important *via* food containers [5–7]. Phthalates are used as liquid plasticizers found in a wide range of products including plastics, coatings, toys, cosmetics, and medical tubing. They are classified in two groups: high molecular weight phthalates, such as diethylhexyl phthalte (DEHP), and low molecular weight phthalates, such as dibutyl phthalates (DBP) [8]. DDT, an organochlorine pesticide, was largely used after the Second World War for its insecticidal properties. Although it was banned in the 1970s in the Western World, it continues to be used in developing countries. DDT is a synthetic mixture of three isoforms: p,p'DDT, o,p'DDT and p,p'DDD. EDCs are originally thought to act through nuclear hormone receptors, such as estrogen receptor (ER) or androgen receptor (AR) [9]. During the last decade, we, and others, were interested in the effect of EDCs on G-protein-coupled hormone receptors (GPCRs). These studies have shown that there are chemical compounds in the environment capable of binding to GPCRs and disrupting the activity and intracellular signaling pathways of receptor. Moreover, EDCs may alter pathways involved in hormone biosynthesis and/or receptor signaling regulation. This review summarizes the effects of three classes of EDCs on hormones homeostasis and GPCRs signaling involved in the HPG axis. Several molecular mechanisms can be involved in the EDC effects on the HPG axis. All studies cited here were performed in human species.

2. GPCRs implicated in the HPG axis

The GPCRs are the largest family of cell-surface receptors with over 800 members accounting for 4% of the encoded human genome [10]. About half of them have sensory functions, mediating olfaction, taste, light perception, and pheromone signaling. The other half (~350–400) are called endo-receptors, i.e. receptors that interact with endogenous ligands [11]. These receptors are involved in the detection of many extracellular stimuli (from photons or ions to large hormones proteins). Thus, they have important roles in various physiological systems. Dysfunction of GPCRs contributes to many human diseases and GPCRs represent 34% of all Food and Drug Administration-approved drugs [12].

GPCRs are characterized by a common structure with seven transmembrane helices with an extracellular N terminus and an intracellular C terminus [13]. The N-terminal portion, or transmembrane domain, constitute the ligand binding site while the C-terminal portion and the intracellular loops form a coupling domain with the intracellular effectors [14].

In the classical GPCR signaling pathway, after ligand binding, activated-GPCR binds the intracellular heterotrimeric G proteins, promoting the release of GDP from the Gα subunit, exchanged for GTP and the dissociation of the GTP-bound α subunit from $\beta\gamma$ dimers. The activated G proteins can then transduce and amplify GPCR signals via second messengers to produce a variety of cell responses [15]. Briefly, Gαs activates adenylyl cyclases to catalyze the conversion of ATP to cAMP. Members of the Gαi family primarily inhibit cAMP production. The G$\alpha_{q/11}$ family converts phosphatidylinositol 4,5-bisphosphate to diacylglycerol and inositol 1,4,5-trisphosphate to activate Protein Kinase C and increases intracellular Ca^{2+} levels. Approximately 10% of GPCRs can be coupled with different types of Gs subunit depending on cell type and context [16]. The second messengers then target other enzymes such as cAMP-dependent protein kinase A (PKA), GMP-dependent protein kinase G (PKG), Ca^{2+}-dependent protein kinase C (PKC) or calcium-sensitive enzymes. The G$\beta\gamma$ subunit can also activate a multitude of effectors (GRKs, ion channels, PI3K, phospholipases, MAP kinases) to induce a variety of

cellular effects [17]. G protein-mediated signaling is discontinued when the Gα subunit hydrolyzes GTP to GDP, due to its intrinsic GTPase activity. This then leads to the reassociation of Gα with Gβγ to form the inactive heterotrimer [14]. In addition to canonical signaling through heterotrimeric G proteins, some of GPCRs can use alternative modes of GPCR activation and initiate G protein–independent pathway. The main independent pathway involves a coupling with β-arrestin. Originally, β-arrestin was identified as an essential factor in the endocytosis and arrest of GPCR signaling induced by heterotrimeric G proteins. Today, other functions associated with β-arrestins are being studied and coupling to β-arrestins is increasingly described as "scaffolding" proteins involved in multiple G protein-independent signaling pathways. Indeed, in addition to clathrin, β-arrestins are able to bind to many proteins involved in different signaling pathways (Src, ERK1/2 and JNK3 kinases protein phosphatases, ubiquitin ligases...) [18]. The activation of β-arrestins signaling pathways can take place at the membrane but also in intracellular after internalization [15]. Indeed, a growing amount of evidence suggests that several molecules have not been known to be regulated by G proteins, suggesting that β-arrestin-mediated signaling pathways may be functioning in parallel with G-protein-mediated pathways enhancing GPCR signaling pathways.

The Hypothalamo-Pituitary-Gonadal axis is active in the midgestational fetus and after birth at the minipuberty but is mainly reactivate at onset of puberty. Some receptors of the HPG axis belong to the subfamily of GPCR: gonadotropin-releasing hormone receptor (GnRHR), GPR54/Kisspeptin receptor, Neurokinin B receptor (NK3R), Prokineticin receptor (PROKR2), follicle stimulating hormone receptor (FSHR), human chorionic gonadotropin/luteinizing hormone receptor (hCG/LHR) and Relaxin Family Peptide Receptor 2 (RXFP2).

The GnRH, a neuropeptidic hormone, is secreted by hypothalamic GnRH-expressing neurons into the portal blood vessels in rhythmic pulses [19]. It binds to a membrane receptor, the GnRH receptor, also known as the luteinizing hormone releasing hormone receptor (LHRHR), on pituitary gonadotropic cells and stimulates the biosynthesis and secretion of LH and FSH [19]. GnRHR is predominantly coupled to the Gq-protein [20]. GnRH/GnRHR pathway constitutes the initial step in the HPG axis and controls reproduction in both sexes. GnRH loss-of-function mutations are associated to normosmic hypogonadotropic hypogonadism [21]. GnRH neurons appear to be directly regulated by Kisspeptin-1 (KISS1), with Neurokinin B (NKB) and Prokineticin 2 (PROK2). KISS1 is a peptidic hormone mostly expressed in the hypothalamus [22]. It activates GPR54/KISS1R, which results in the activation of phospholipase C *via* Gq [12]. GPR54 has been described in brain regions, including hypothalamus, but also in peripheral regions [22]. Kisspeptin/GPR54 pathway has a crucial role in the onset of puberty, the regulation of sex hormone mediated secretion of FSH/LH, and in the control of fertility [22, 23]. Inactivating and activating mutations in *KISS1* or *GPR54* genes have been associated with hypogonadotropic hypogonadism and precocious puberty, respectively [23].

Gonadal function is under pituitary control *via* the gonadotropin hormones: follicle stimulating hormone (FSH) and luteinizing hormone (LH) [24]. FSH and LH are synthesized and secreted by the pituitary gonadotropic cells and work together in the reproductive system. The human chorionic gonadotropin (hCG) is secreted by the placenta and controls ovarian function during gestation. LH and hCG share the same GPCR, the hCG/LHR. The FSH and hCG/LH receptor belong to the glycoprotein-hormone receptor family. Activation of the LH and FSH receptor results in the production of intracellular cyclic AMP (cAMP) *via* $G\alpha_s$ proteins [25, 26]. However, FSHR and LHR can also couple to several other effectors such as $G\alpha_q$ and β-arrestin [26–28]. FSHR is expressed in Sertoli and granulosa cells in male

and female gonads, respectively, and is required for normal spermatogenesis and growth and maturation of ovarian follicles, as well as for estrogen production [29]. In women, LHR induces luteinization of granulosa cells, progesterone synthesis and *corpus luteum* maintenance during the luteal phase [30]. In men, LH stimulates testosterone production by Leydig cells [30].

Steroid hormones (estrogen, progesterone, and testosterone) secreted by the gonads, bind, and activate nuclear receptors. However, a membrane associated estrogen receptor (GPER) has been identified 15 years ago [31, 32]. Activation of GPER induces intracellular calcium mobilization, cAMP production and phosphorylation cascade involving $ERK_{1/2}$, PKA, PI3K [33]. This receptor is implicated in many physiological functions: uterine proliferation, metabolism, cardiovascular, immune, and neural system.

More recently, the INSL3/RXFP2 system pathway was identified for its role in reproduction. Insulin-like peptide-3 (INSL3) belongs to the insulin/relaxin family of peptidic hormones [34, 35]. This hormone is mainly produced by testicular Leydig cells and the production is dependent on the state of Leydig cell differentiation [34]. INSL3 is considered as a marker for Leydig cells function. Its best characterized role is in the control of testicular descent since *INSL3* gene inactivation males have bilateral cryptorchidism with testis remaining in abdominal position [36, 37].

3. Effects of EDCs on signaling of HPG axis G-protein coupled receptors

Effects of EDCs on the activity of HPG axis GPCR identified in the literature search are summarized in **Table 1**.

3.1 Hypothalamic hormones receptor

Currently, there are no data on the effects of EDCs on the activity of human hypothalamic hormone receptors. However, some studies have been conducted with animal models. Exposure to phthalates leads to a modulation of GnRHR expression (positive or negative depending on the studies) [50, 51], as well as an increase in its expression in rat uterus [52].

3.2 Gonadotropin hormones receptor

EDCs, like phthalates, increase the FSHR expression in human granulosa cells [38]. DDT has been shown to disturb the FSH induced-cAMP accumulation [39] and aromatase activity in human granulosa cells [40]. Recently, we showed that DDT behaves as an FSHR positive allosteric modulator [41]. DDT interacts with the receptor in the minor binding pocket in the transmembrane domain. DDT acts on the early steps of activation of the FSHR and induces an increase in FSH-stimulated cAMP production. Moreover, the binding of DDT enhances the FSHR response to hCG. The increased response to FSH in the presence of DDT and the gain of sensitivity to hCG may therefore by deleterious. In opposite, BPA is a FSHR negative allosteric modulator [41].

As for FSHR, EDCs, like BPA, disturbes the expression of hCG/LHR in human endometrial stromal cells [42]. In CHO-K1 cells stably transfected with hCG/LHR, DDT reduced the cAMP accumulation induced by hCG [39, 41] and hLH (Munier et al., Arch Toxicol, in revision). Moreover, DDT decreases the hCG- and hLH-promoted β-arrestin 2 recruitment (Munier et al., Arch Toxicol, in revision). DDT seems to act as a negative allosteric modulator of the hCG/LHR signaling.

GPCR	EDC	Study model	Main results	
FSHR	DBP $[10^{-7}$ to 10^{-4} M]	Human granulosa cells	DBP increases FSHR expression	[38]
	DDT $[10^{-7}$ to 10^{-4} M]	CHO-K1 - hFSHR	DDT decreases cAMP production stimulated by FSH	[39]
	DDE $[10^{-7}$ to 10^{-4} M]	Human granulosa cells from IVF	DDE potentiates the FSH induced aromatase activity	[40]
	DDT $[10^{-7}$ to 10^{-5} M]	CHO-K1 - hFSHR	DDT is an FSHR positive allosteric modulator	[41]
	BPA $[10^{-5}$ M]	CHO-K1 - hFSHR	BPA decreases cAMP production stimulated by FSH	[41]
hCG/LHR	BPA $[10^{-6}$ M]	Human endometrial stromal cells	BPA decreases hCG/LHR expression	[42]
	DDT $[10^{-7}$ to 10^{-5} M]	CHO-K1 - hCG/LHR	DDT decreases cAMP production stimulated by hCG/LHR	[41]
RXFP2	DEHP $[10^{-9}$ to 10^{-5} M]	HEK293 - hRXFP2	DEHP increases cAMP production stimulated by INSL3	[43]
	DBP $[10^{-9}$ to 10^{-5} M]		DBP increases cAMP production stimulated by INSL3	
	BPA $[10^{-11}$ to 10^{-7} M]	HEK293 - hRXFP2	BPA increased cAMP production stimulated by INSL3	
	DEHP + DBP + BPA $[10^{-10}$ to 10^{-6} M]	HEK293 - hRXFP2	DEHP+DBP + BPA mixture decreases cAMP production stimulated by INSL3	
GPER	BPA $[10^{-6}$ M]	Human breast cancer cells	BPA increases GPER expression	[44]
	BPA $[10^{-12}$ to 10^{-9} M]	Human testicular seminoma cells	BPA promotes cellular proliferation *via* GPER activation	[45]
	BPA $[10^{-9}$ to 10^{-5} M]	HEK293 - hGPER	BPA is a GPER agonist and induces the Gs protein pathway	[46]
	BPA $[10^{-9}$ to 10^{-5} M]	Human breast and lung cancer cells; cancer-associated fibroblasts	BPA induces ERK$_{1/2}$ activation and gene expression through GPER leading to cellular proliferation and migration	[47, 48]
	BPA $[10^{-7}$ to 10^{-4} M]	Human granulosa cells	BPA induces apoptosis *via* GPER activation	[49]
	o,p'-DDE $[10^{-7}$ to 10^{-6} M]	Human breast cancer cells	o,p'-DDE is a GPER agonist and induces the Gs protein pathway	[32, 46]

Table 1.
Experimental studies studying the effect of EDC on HPG axis GPCR signaling.

3.3 Insl3 receptor, RXFP2

Only one study has very recently focused on the effect if EDCs on receptor signaling to INSL3: RXFP2. In a cellular model of HEK293 transiently expressing human RXFP2, individually, BPA, DEHP and DBP potentiate the cAMP response to INSL3 [43]. Because of their ubiquity, BPA, DEHP and DBP are present in many human biological fluids, as the amniotic liquid. Furthermore, everyone is chronically exposed to mixtures of environmental chemical factors resulting in toxicological interactions that cannot be predicted by reprotoxicological studies of single molecules. The combination of these three molecules, at concentrations found in human amniotic fluid, decreases the basal activity of RXFP2 as well as the response to INSL3. The structural similarity between FSHR and RXFP2 suggests that small hydrophobic molecules, like phthalates and BPA, could use the same binding sites as DDT in FSHR. The binding of one or two compounds to this site could lead to a stabilization of the active state of the receptor driving an increase of agonist activity [53]. In contrast, the binding of three compounds (DEHP+DBP + BPA) likely leads to a steric hindrance that may prevent the conformational changes necessary for the activation of RXFP2 and probably stabilize an inactive state. This study shows that in addition to individual EDC targets, HPG axis GPCRs can also be targeted by EDC cocktails.

3.4 Membrane sexual steroid hormones receptor

The G protein-coupled receptor (GPER/GPR30) is a membrane estrogen receptor [31]. Gene inactivation of *GPER* in mice did not induce major modifications in reproductive function [54]. However, several studies show that this receptor has pro-oncogenic effects in hormone-dependent cancers. Although many EDCs exhibit low binding affinities to the nuclear ERs and often require relatively high concentrations (>1 μM) to affect genomic pathways, several studies have focused on non-genomic signaling mediated by GPER [55].

Various DDT derivatives and BPA bind to GPER with a K_d between 1 to 10 μM and are competitors of E2 [46]. The binding affinity of EDCs for GPER is higher than for the nuclear receptors. Nevertheless, low concentrations of o,p'DDE and BPA increased cAMP production by GPER [32, 45, 46]. BPA and phthalate (MEHP) also affect proliferation and migration in human cervical cancer cells [56], in human seminoma cells [45], human breast cancer cells and cancer-associated fibroblasts that lack nuclear ERs [47, 57] as well as the migration and invasion of lung cancer cells [48]. BPA modifies these cellular responses by modulating different intracellular signaling pathways ($ERK_{1/2}$ or Akt phosphorylation, gene expression) through GPER activation. In opposite, GPER mediates BPA-induced intracellular stress generation (ROS production and calcium accumulation) and apoptosis (caspase activation and mitochondrial membrane potential decrease) in human granulosa cells [49]. Recently, it has also been shown that BPA increases GPER gene expression in breast cancer cell lines [44]. Finally, bisphenols AF and B, two substitutes of BPA, exert high estrogenic effects via GPER pathway at nanomolar concentrations [58, 59].

4. Effects of EDCs on the synthesis and secretion of HPG axis hormones

Effects of EDCs on the synthesis and secretion of HPG axis hormones identified in the literature search are summarized in **Table 2**.

Study population	EDC exposure	Matrix/ biomarker	Main results	
192 mother–child pairs from e-waste recycling town and 70 from control area	Free BPA in cord blood serum	*Kiss* gene expression in placenta	Higher BPA concentrations showed positive correlation with *Kiss* gene expression	[60]
73 girls with central precocious puberty and 31 controls	Seven urinary phthalate metabolites concentrations	Serum kisspeptin	Positive correlation between kisspeptin- and mono-n-butyl phthalate	[61]
535 men (18–40 yr) living or not in pesticides contamined area.	Lipid-adjusted DDE and DDT concentrations	Serum FSH, LH, T, E2	Positive association between DDT or DDE with T	[62]
749 Swedish (fishermen and their pregnant wife)	p,p′-DDE serum level	Serum FSH, LH, T, E2	Positive association between DDE and FSH or LH	[63]
97 adult men living in nothern Thailand	plasma levels of DDT and its metabolites	Serum FSH, LH, T, E2	Negative association of E2 level with p,p'-DDE and positive association with o,p'-DDE	[64]
107 males exposed to DDT in Italy	Lipid-adjusted p,p'-DDE and p,p'-DDT serum concentration	Serum FSH, LH, T, E2	No association with serum hormone levels	[65]
604 adults (men and women) in Brazil areas exposed to pesticides	Serum concentrations of 19 pesticides including p,p'-DDT and o,p'-DDT	Serum FSH, LH, T, E2	In men, o,p'-DDT level was associated with lower T, in peri- and postmenopausal women, p,p'-DDT showed inverse associations with LH; No association in premenopausal women	[66]
234 mothers and their sons	Serum o,p'- and p,p'-DDT, p,p'-DDE from mothers during pregnancy or at delivery and their sons at 9 years.	Serum FSH, LH and T in sons at 12 years	Prenatal maternal DDT and DDE levels were associated with decreases in LH	[67]
45 girls with early breast development, 16 girls with early puberty, and 33 girls with no signs of puberty	2,4-DDT and 4,4'-DDE in the serum and adipose tissue samples.	Serum basal and stimulated LH and FSH level	Basal and stimulated LH were higher in girls with detectable serum DDE levels	[68]
308 young men	Urinary BPA concentrations	Serum LH, T, E2	Higher urinary BPA concentrations were associated with increased serum T, E2, and LH	[69]
215 healthy young men (18–23 yr)	Urinary BPA concentrations	Serum FSH, LH, T, E2	Positive association between urinary BPA and LH levels	[70]

Study population	EDC exposure	Matrix/ biomarker	Main results	
560 men aged 18–55 years	Urinary BPA concentrations	Serum FSH, LH, T	BPA was associated with increased serum levels of LH and FSH in male smokers, and with decreased serum levels of total T in men with BMI ≥ 25 kg/m2.	[71]
167 men from an infertility clinic	Urinary BPA concentrations	Serum FSH, LH, T, E2	Positive association between urinary BPA and serum FSH	[72]
244 mothers-child pairs	Serum maternal total BPA concentration during second or third trimester	Serum FSH, LH, T, E2	No association with serum hormone levels	[73]
159 women with premature ovarian insufficiency and 186 controls	Urinary concentrations of BPA	Serum FSH, LH	No association with serum hormone levels	[74]
106 BPA-exposed factories and 250 unexposed female workers	Urinary concentrations of BPA	Serum FSH, LH, E2	Inverse association between BPA and FSH in unexposed group	[75]
143 healthy, premenopausal women	Urinary concentrations of BPA	Serum FSH, LH, E2	No association with serum hormone levels	[76]
172 peripubertal boys	Urinary concentrations of BPA	Serum FSH, LH, T	No association with serum hormone levels	[77]
130 children with Attention-Deficit/ Hyperactivity Disorder and 68 controls (boys and girls)	Urine levels of phthalates and BPA	Serum FSH, LH, T, E2	Among boys with ADHD, MBzP and MEHP levels were positively correlated with T; among girls, MEP was positively correlated with LH and T	[78]
136 girls (6–9 yr) with early puberty and 136 controls	Urinary BPA concentrations	Serum basal and stimulated LH and FSH level, E2	In early puberty group, negative correlation between BPA and peak FSH levels	[79]
479 pregnant women and their infants (boys and girls)	Urinary 12 phthalate metabolites concentrations at gestational week 28	Serum T, LH, FSH during mini puberty	No association with serum hormone levels	[80]
302 Korean children and adolescents	Urinary and serum concentrations of DEHP, MEHP, DBP, MBP	Serum FSH, LH, T, E2	Positive correlations between serum DBP or MEHP, and E2 and/or LH in children.	[81]

Study population	EDC exposure	Matrix/ biomarker	Main results	
106 males and females (11–88 yr)	Urinary phthalate metabolites	Serum FSH, LH, T, E2	Positive associations between MEHP and FSH or T, MEOHP and FSH, LH or T, negative associations between MEHHP and LH, FSH or T	[82]
88 infertile men	Urinary and serum concentrations of 11 phthalate metabolites	Serum FSH, LH, T	Negative associations between FSH and MiBP and MCMHP; positive association between T and phthalates metabolites.	[83]
599 infertile men	Urinary concentrations of 8 phthalate metabolites	Serum FSH, LH, T, E2	Inverse associations between T and MiBP, FSH and MEHHP, positive relationship between E2 and MEP, %MEHP and FSH and LH	[84]
295 adult men	Urinary concentrations of phthalate metabolites	Serum FSH, LH, T, E2	Negative association between MBzP and FSH	[85]
881 healthy men	Urinary concentrations of 14 phthalate metabolites	Serum FSH, LH, T, E2	%MEHP was negatively associated with T and FSH	[86]
Male with cryptorchidism (421), hypospadias (109) or controls (425)	5cx-MEPP, 7cx-MMEHP in amniotic fluid (11–21 weeks)	INSL3, T in amniotic fluid	Negative correlations between INSL3 and cx7-MMeHP and 5cxMEPP	[87]
1066 Chinese men of reproductive age	Urinary concentrations of 14 phthalate metabolites	Serum levels of INSL3, FSH, LH, T	Negative association between INSL3 and MEHP; negative association between MBP and MiBP with T and LH	[88]
male partners of subfertile (n = 253) and fertile (n = 37) couples	11 phthalate metabolites in urine and semen	Serum levels of INSL3, FSH, LH, T, E2	Negative association between INSL3 and some urinary and seminal phthalate metabolites	[89]
case–control study of 176 men (fertile and infertile)	Urinary concentrations of 11 phthalate metabolites	Serum levels of INSL3, FSH, LH, T, E2	inverse association MMP, MiBP, MEHP, MEHP% and T; MBzP and MEHP% were negatively associated with serum INSL3 level	[90]
102 mother–child pairs	Maternal serum concentration of MEHP (23–35 weeks of gestation	Cord blood INSL3, FSH, LH, T, E2 levels	Inverse associations between maternal MEHP and INSL3 in males	[91]

Study population	EDC exposure	Matrix/ biomarker	Main results	
52 boys with cryptorchidism and 128 control boys	Cord blood BPA concentration at birth	Cord blood INSL3 levels	Higher cord blood BPA concentrations were associated with reduced cord blood INSL3 levels	[92]

T: testosterone, E2: estradiol.

Table 2.
Human biomonitoring studies addressing the relationship between EDC and hormones of HPG axis.

4.1 Hypothalamus level

4.1.1 Kisspeptin

No data are available on the impact of DDT on Kisspeptin in epidemiological studies in humans.

Interestingly, a study led on 262 mother–child pairs from China found a positive correlation between cord blood levels of BPA and *KISS1* mRNA expression in placenta tissue [60].

For phthalates, linear regression analysis showed increasing trend for kisspeptin secretion with the concentration of urinary phthalates [61].

4.1.2 GnRH

No epidemiological or experimental studies are available on the possible link between EDC levels and GnRH concentration in human. This is probably explained by the pulsatile nature of its release and the lack of dosage in clinical practice. However, many effects of EDC on GnRH were observed in rodents [93].

4.2 Pituitary level

DDT is rapidly metabolized in the body to DDE. Thus, in epidemiological studies, DDE is dosed in the blood more often than DDT. In a cohort of men of reproductive age, statistically significant positive association was found between the serum level of DDE and LH or FSH [63]. However, others studies did not reveal any association between DDT and FSH or LH levels in adult men [62, 64, 65]. In peri and postmenopausal women, inverse correlation was found between serum DDT and LH [66]. Moreover, it has been shown that maternal exposure to DDT or DDE, assayed in prenatal serum, induced a reduction of plasma LH in teenage boys, not found for FSH [67]. A study also showed that the serum levels of LH (basal level and after GnRH stimulation) was significantly higher in girls with detectable serum DDE levels than in girls with undetectable DDE [68]. This difference was not found for FSH [68].

For BPA, studies found that higher urinary BPA concentration was associated with significantly higher concentrations of serum LH in healthy young men, with or without association with FSH [69–71]. However, these results were not confirmed in others cohorts of fertile men [73]. Conversely, another study found a positive correlation between urinary BPA concentration and FSH level, without change in LH level in a cohort of infertile men [72]. In women, no association was found between urinary bisphenol A and LH or FSH levels in premenopausal women [74–76]. Moreover, no

association was found in healthy children for LH and FSH [77, 78]. A modest negative correlation was found between urinary BPA concentration and peak of GnRH-stimulated FSH levels in girls with idiopathic central precocious puberty, without difference for LH levels [79].

Maternal phthalates exposure (urinary samples collected during second trimester) was not associated with serum LH level or FSH in offspring during mini-puberty in boys and girls [80]. However, positive correlations were observed between different phthalates and serum LH in prepubescent Korean children (for serum DBP or MEHP) [81], in girls with attention-deficit/hyperactivity disorder (for urinary MEP) [78] and in Chinese population (11–88 years, males and females) (for urinary MEHHP levels) [82]. In the same populations, either negative [82, 83] or no effects [78, 81] were observed on FSH level. In men, urinary phthalate metabolites were positively associated with LH and FSH levels [84] in one study while negative association between urinary phthalates concentrations and levels of FSH was found in American men (for MBzP) [85] and in Danish men (for MEHP or %MiNP) [86] without impact on LH.

Altogether, epidemiological data have linked exposure to EDC and LH and/or FSH level but evidence were often inconclusive. The inconsistent findings may partly be due to differences in the characteristics and sizes of the cohorts and to the different EDC exposure levels among studies.

4.3 Gonadal level

4.3.1 Sexual steroid hormones

Many data are already available on the effect of endocrine disruptors on the secretion of sex steroids. Recent reviews list all available studies for DDT [93], BPA [93, 94] or phthalates [93, 95–97].

4.3.2 INSL3

No data are available on the impact of DDT on INSL3 in humans epidemiological studies.

Several studies showed that INSL3 was negatively impacted by putative phthalate metabolites. The Diisononyl phthalate (DiNP) metabolite, cx7-MMeHP, and the DEHP metabolite, 5cxMEPP, showed significant negative correlations with INSL3 in amniotic fluid for weeks 11–22 [87]. Moreover, serum levels of INSL3 was negatively associated with urinary concentration of mono-2-ethylhexyl phthalate (MEHP) and MBzP among large cohorts of chinese men of reproductive age [88–90]. In adjusted models, quartiles increases in phthalates metabolites correlated with significant decreases in plasma INSL3 levels [88–90]. It has also been shown that maternal serum MEHP concentration (from 23–35 weeks of gestation) was negatively correlated with INSL3 level in cord blood mainly in boys [91].

There is also an inverse correlation between BPA level and concentration of INSL3 [92]. Indeed, in a population of 180 boys born after 34 weeks of gestation (52 cryptorchid and 128 control), cord blood levels of free BPA correlated negatively with INSL3 [92]. In this study, cord blood INSL3 level was also significantly decreased in the cryptorchid group compared with the control group [92].

Ex vivo studies on human testicular explant were performed, to study more precisely the effect of endocrine disruptors on the secretion of INSL3.

No data are available on the impact of DDT on INSL3 in humans experimental studies.

The exposure of fetal testis (8–12 weeks) to BPA at 10^{-8} M and 10^{-5} M for 72 h [98], significantly depressed the basal INSL3 production compared with control. This treatment also reduced INSL3 mRNA level by more than 20% [99]. However, BPA did not modify hCG or hLH-stimulated INSL3 production [98]. Conversely, in human adult testes, BPA increased significantly INSL3 production by Leydig cells, at a low doses (10^{-9} M) [100]. Interestingly, its analogs, Bisphenol B and Bisphenol S also increased INSL3 production at 10^{-9} and 10^{-8} M. Moreover, BADGE, another bisphenol, dose dependently increased INSL3 after 48 h of exposure. In contrast, BPE dose dependently inhibited INSL3 levels [100].

For phthalates, di-(2-ethylhexyl) phthalate (DEHP) and mono-(2-ethylhexyl) phthalate (MEHP) exposition on organo-cultured adult human testis did not affect Leydig cell INSL3 concentrations [101].

5. Conclusions

Most epidemiological and experimental studies focus on the effect of EDCs on the expression and secretion of hormones, as well as on the activity of nuclear steroid receptors. However, a few experimental studies have shown that G protein-coupled membrane receptors of the HPG axis are targets of EDCs as well. It can be pointed out that most of the studies analyzing the effects of EDCs on GPCRs of HPG axis have been performed with cell culture systems. *In vitro* models are valuable tools because they are easily manipulated. But the comparison of the effects of EDCs in wild-type and GPCRs- inactivated animal models could provide additional informations on the mode of action of these compounds.

Mechanisms of GPCR disruption by EDCs include: (1) changes in the expression; (2) interaction with transmembrane domain receptor; (3) modulation of intracellular signaling pathways.

The GPCRs of HPG axis, involved in diverse physiological functions, should be considered as possible contributors of the adverse effects of EDCs on reproduction. How their modulation by EDCs contributes to these deleterious effects should be an important field of investigations in the near future.

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Conflict of interest

The authors declare no conflict of interest.

Author details

Valentine Suteau[1,2], Patrice Rodien[1,2,3] and Mathilde Munier[1,2,3]*

1 UMR CNRS 6015, INSERM 1083, University of Angers, France

2 Department of Endocrinology, University Hospital, Angers, France

3 Reference Center for Rare Diseases of Thyroid and Hormone Receptors, Angers, France

*Address all correspondence to: mathilde.munier@univ-angers.fr

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