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Nuclear receptors are the major targets of endocrine disrupting chemicals

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Abstract

Endocrine disrupting chemicals (EDCs) are exogenous substances that are suspected to cause adverse effects in the endocrine system mainly by acting through their interaction with nuclear receptors such as the estrogen receptors α and β (ER α and ER β), the androgen receptor (AR), the pregnan X receptor (PXR), the peroxisome proliferator activated receptors α and γ (PPAR α , PPAR γ) and the thyroid receptors α and β (TR α and TR β). More recently, the retinoid X receptors (RXR α , RXR β and RXR γ), the constitutive androstane receptor (CAR) and the estrogen related receptor γ (ERR γ) have also been identified as targets of EDCs. Finally, nuclear receptors still poorly studied for their interaction with environmental ligands such as the progesterone receptor (PR), the mineralocorticoid receptor (MR), the glucocorticoid receptor (GR), the retinoic acid receptors (RAR α , RAR β and RAR γ), the farnesoid X receptor (FXR) and the liver X receptors α and β (LXR α and LXR β) as well are suspected targets of EDCs. Humans are generally exposed to low doses of pollutants, therefore the aim of current research is to identify the targets of EDCs at environmental concentrations. In this review, we analyze recent works referring that nuclear receptors are targets of EDCs and we highlight which EDCs are able to act at low concentrations.

1 Introduction

Since industrialization with the objective to improve the quality of human lives in any aspects outgrew into global dimension, multitude compounds with endocrine activity coined endocrine disrupting chemicals (EDCs), specifically bisphenols (BPs), alkylphenols, phthalates, organochlorins, polychlorinated biphenyls, perfluoroalkyl compounds and natural phyto- and mycoestrogens, were introduced into environment. Indeed, the environment became “a matrix” for bioaccumulation of persistent chemicals commonly utilized in plastic

bottles, food packaging, flame retardants, pesticides, cosmetics, medical devices and drugs, toys, textiles, carbonless receipts, water pipes and plenty of other daily used man-made products. The contamination is even more emphasized by their migration and substantial oral (consuming fresh food containing phytoestrogens or contaminated with organochlorines), dermal exposures (thermal paper, cosmetics) or inhalation (dust, fumes). Such daily exposition along to pervasive and bio-accumulative character of these environmental toxicants have been linked to a wide range of adverse health outcomes manifesting as consequent reproductive, developmental, metabolic, neurological diseases and even hormone-related cancers (Giulivo et al, 2016; Gore et al, 2015; Schug et al, 2011; Zoeller et al, 2014).

EDCs are capable to interfere with the synthesis, transport, action and metabolism of hormones. Indeed, the main known targets of EDCs actions are nuclear receptors (NRs) (Delfosse et al, 2015a; Diamanti-Kandarakis et al, 2009; Swedenborg et al, 2009). There are 48 members of the NRs superfamily in humans, 24 members have established ligands and the remnant 24 are classified as “orphans” or “adopted orphans” with latter identified ligands. These ligand-inducible, transcription modulating proteins comprise canonical domain structure: a variable, intrinsically unfolded N-terminal A/B domain harboring transcriptional activation function 1 (AF-1), a conserved DNA-binding domain (DBD), and a C-terminal ligand-binding domain (LBD) hosting activation function 2 (AF-2) (Weikum et al, 2018). Predominantly hydrophobic pocket enclosed in the LBD can accommodate plenty of ligands, small lipophilic endo- as well as exogenous compounds of micro-/nanomolar affinities.

The first unveiled targets of EDCs were the estrogen receptors α and β (ER α and β , NR3A1 and NR3A2) and the androgen receptor (AR, NR3C4) whiles the other steroid receptors, the glucocorticoid receptor (GR, NR3C1), the mineralocorticoid receptor (MR, NR3C2) and the progesterone receptor (PR, NR3C3) were less studied. These receptors reside in their monomer *apo* forms in the nucleus (ERs) or are present in the cytoplasm (AR, PR, GR, MR) complexed to chaperones. Then, agonist binding triggers a conformational change that leads to the dissociation of chaperones, nuclear translocation of monomer, homodimer formation, binding to specific response elements (RE) in DNA and finally, transcriptional regulation of target genes (Dahlman-Wright et al 2006; Lu et al, 2006). Other well documented EDCs targets belong to the retinoid X receptor (RXR α , RXR β and RXR γ , NR2B1, NR2B2 and NR2B3) heterodimer family; the pregnane X receptor (PXR, NR1I2), the peroxisome proliferator activated receptor α and γ (PPAR α and PPAR γ , NR1C1, and NR1C3) and the thyroid hormone receptors α and β (TR α and TR β , NR1A1 and NR1A2) (Balaguer et al, 2017; Delfosse et al, 2015a; le Maire et al, 2010; Zoeller 2005). Less NRs studied within this family are the constitutive androstane receptor (CAR, NR1I3), the retinoid acid receptors (RAR α , RAR β and RAR γ , NR1B1, NR1B2 and NR1B3), the liver X receptors α and β (LXR α and LXR β , NR1H3 and NR1H2) and the farnesoid x receptor (FXR, NR1H4). In absence of

ligands, these receptors are bound in heterodimeric form with RXR to specific REs on DNA in association with corepressors. In presence of agonist, corepressors dissociate, coactivators are recruited and transcription of target genes is launched (Germain et al, 2002; Leblanc et Stunnenberg, 1995; Willy et al, 1995). Finally, among the orphan receptors, the estrogen-related receptor γ (ERR γ , NR3B3) activity can be modulated by several exogenous compounds (Li et al, 2010; Tohme et al, 2010). In this review, we analyze recent works referring the known NRs targets of EDCs. As well, we discuss the possibility that other NRs emerge as potential targets of these compounds.

2 Steroid receptors

2.1 Estrogens receptors

ER α and ER β are receptors for the natural estrogen 17 β -estradiol (E2), playing important roles in the growth and maintenance of a diverse range of tissues such as the mammary gland, uterus, bone or the cardiovascular system. Furthermore, estrogens are the key regulators of primary breast, endometrial and ovarian cancer growth. Both ERs are widely distributed throughout the body, displaying distinct but overlapping expression patterns in a variety of tissues (Couse and Korach, 1999). Although ER α and ER β share similar action mechanisms, several differences in the transcriptional abilities of each receptor as well as distinct phenotypes among gene null animals have been identified, suggesting that these receptors regulate distinct cellular pathways (Couse and Korach, 1999).

Interestingly, ER β has been shown to antagonize the effects mediated by ER α in many tissues (Docquier et al. 2013; Sotoca et al, 2008). In the breast cancer cell lines, overexpression of ER β inhibits E2-mediated proliferation and gene expression (Williams et al. 2008). In the prostate similarly, ER α promotes cell proliferation and survival, while ER β is protective and pro-apoptotic (Nelson et al. 2014). The example of their *in vivo* antagonism is observed in mice behavior; ER β KO mice have an increase in sexual aggression compared to WT mice (Ogawa et al. 1999). At the molecular level, ER α and ER β signal in opposite ways (activation vs. repression of transcription) at an AP1 site (Paech et al. 1997). In this regard, EDCs with ER-subtype selectivity may produce different biological outcomes, particularly on cancer cell proliferation.

ERs are rather promiscuous nuclear receptors considering the size of ligand binding pockets accommodating various exogenous, natural or synthetic compounds (xenoestrogens), as well as number of industrial chemicals. The natural endogenous estrogens (estradiol, estriol, estrone) are high affinity ligands of ERs with individual dissociation constants (Kds) in the sub-nanomolar range. Contrary, the xenoestrogens such as the phytoestrogens genistein and ferutinin, the pesticide metabolites DDE, HPTE and M2 compound (respectively DDT, methoxychlore and vinclozolin metabolites), the plasticizers bisphenols A and C, the phthalate

benzyl butyl phthalate, the benzophenone 2 and the propyl and butyl parabens used as UV filters and preservatives, respectively, bind to ERs with affinities in the sub- to micromolar range concentrations (Delfosse et al, 2012; 2014), far above the environmental values. All of them bind to the hormone-binding site of ERs and are engaged in different sets of ligand/protein interactions according to their size and chemical structures. The small-size compounds enabling fewer contacts with the receptor cavity are generally associated with lower binding affinities whereas larger EDCs adopting the binding mode reminiscent of that used by the endogenous ligands are characterized by higher interaction capacities. This is the case of mycoestrogens, α -zearalenol (α -ZA) and its derivatives that bind and activate ERs with high affinity (Delfosse et al, 2014) (table 1). Some of EDCs display subtype selectivity among ERs. For example, phytoestrogens like genistein and liquiritigenin bind ER β with 10 fold higher affinity than ER α (Baker et al, 2017; Mersereau et al, 2008). EDCs also display subtype difference in efficacy. Chlordecone, methoxychlore, its metabolite HPTE, bisphenol C and ferutinine were found higher efficacy on ER α than on ER β (Delfosse et al, 2012; 2014). This observation suggests that these EDCs could have stronger impacts on cell proliferation and tumor growth than phytoestrogens as they seem to be more potent on ER α than on ER β .

2.2 Androgen receptor

AR plays the crucial role in the regulation of target genes expression involving physiological processes concerning development and differentiation of male features, (spermatogenesis initiation and maintenance), as well as neuroendocrine system functioning (Matsumoto et al, 2013). Furthermore, androgens are the key regulators of primary prostatic growth. In the absence of ligand, AR is essentially localized in the cytoplasm. Binding to androgens enables heat shock proteins (HSPs) dissociation and AR translocation into the nucleus. Natural androgens (dihydrotestosterone, testosterone, androstenedione) bind AR with affinities ranging in low nanomolar concentrations (Pereira de Jésus-Tran et al, 2006). The presence of androgens is essential for the regulation of male embryo development and differentiation processes as well as spermatogenesis initiation and maintenance. EDCs have been extensively studied for their action on AR and plenty of described estrogenic compounds display also anti-androgenic activities. The most potent of them are α -zearalanol (α -ZA) with its derivatives, the pesticides metabolites M2 compound and DDE, cosmetics like benzophenone 2 and some bisphenols, such as chlorinated BPA and BPC (Delfosse et al, 2012, Molina-Molina et al 2006; 2008; 2013; 2014). The affinities of these diverse environmental antiandrogens are moving in the sub-to micromolar range (table 1).

2.3 Other steroid receptors

As several natural and pharmacological AR ligands are also ligands of the other oxo-steroid receptors, it will not be surprising if some environmental androgens exhibit activities on GR, MR and PR. There are less studies dealing with these receptors compared to that of ERs and AR but bisphenols and pesticides have been characterized their MR, PR and GR antagonistic activity (Grimaldi et al, 2019; Gummy et al, 2008; Kojima et al, 2019; Molina Molina et al, 2006; Rehan et al, 2015; Zhang et al, 2018). Most of these antagonist compounds exhibited low affinity with IC₅₀ in the micromolar range (Table 1). Interestingly, several studies also described such activities in environmental samples (Bellet et al, 2012; Creusot et al, 2014; Zhang et al, 2018) indicating the presence of such compounds at active concentrations.

3. Retinoid X Receptors and their nuclear receptor partners

3.1 Retinoid X Receptors

RXR α , RXR β and RXR γ occupy a central position in the NR superfamily as they are the common heterodimerization partners for one-third of the 48 members of the human NRs including PPARs, PXR, CAR, RARs, FXR, LXRs and TRs. RXR β is widely expressed whereas RXR α and RXR γ expression is more restricted. RXR α is mainly expressed in liver, intestine and skin while RXR γ is the major RXR in the muscle and the brain. RXRs play the key roles in the control of many NR-dependent signaling pathways. RXR heterodimers can be respectively activated by either the partner receptor ligand or the RXR ligand (Le Maire et al 2019). Some heterodimers (PPARs, LXRs, FXR, PXR and CAR) are called permissive because they can be activated by both partner and RXR ligands whereas non permissive heterodimers (TRs, RARs) are only activated by the partner's ligand while RXR is unbound. For all heterodimers, the simultaneous binding of both RXR and partner receptor ligands results in a cooperative and synergistic response (Germain et al, 2002; Evans and Mangelsdorf, 2014). Interestingly, permissive RXR heterodimer partners bind endogenous ligands with low affinity whereas non permissive heterodimers have a strong affinity for their own ligands. This regulatory control of nuclear signaling pathways by multiple RXR heterodimers enables environmental RXR ligands to potentially trigger a multitude of adverse effects on human health.

RXRs are activated by endogenous retinoids such as debated 9-*cis* retinoic acid (9-*cis* RA) (K_d in the nanomolar range) and several other vitamin A metabolites or unsaturated fatty acids (K_ds in the micromolar range). As well, RXRs can be activated by several organotins compounds at nanomolar concentrations (le Maire et al, 2009) (Table 1). Organotins are ubiquitously present throughout the environment due to their widespread use in many industrial and agricultural processes in 1960's. Since 1980's, they were assigned a wide variety of deleterious effects in the endocrine systems of humans and wildlife at even nanomolar concentrations. Interestingly, they do not, neither structurally nor chemically, resemble known NR ligands. The mechanism by which organotins act as endocrine disruptors

has remained enigmatic until the crystal structure of RXR α in complex with tributyltin (TBT) has been solved (le Maire et al, 2009). This structure shows that the organotin occupies only a small part of the ligand binding pocket (LBP) in comparison with 9-*cis* RA. Moreover, it also reveals that the high affinity of TBT for RXRs is derived from the formation of a covalent bond between the tin atom of the organotin and the sulfur atom of conserved LBP cysteine. Although TBT only interacts with only a subset of LBP residues, it is engaged in enough essential contacts to efficiently stabilizing RXR α in its active conformation that is crucial for the recruitment of transcriptional activators. In addition to binding to RXR at very low concentrations, TBT acts as a full agonist activating the receptor as efficiently as 9-*cis* RA. Besides organotins, several EDCs form covalent links with reactive cysteines, thereby increasing their affinity for NRs (our unpublished data).

3.2 Pregnane X receptor and constitutive androstane receptor

PXR is a broad-specificity sensor playing a critical role in the regulation of phase I oxidative enzymes, phase II conjugating enzymes and phase III transport uptake and efflux transporters and co-ordinately regulating steroid, drug, and xenobiotic clearance in the liver and intestine (Orans et al, 2005). Activated PXR binds to gene promoters as a heterodimer with RXR and triggers the expression of target genes such as the cytochrome P450 3A4 (CYP3A4), UDP-glycosyltransferase (UGT1A1), and multidrug resistance protein 1 (MDR1). PXR plays an important role in protecting the endocrine system from EDCs by sensing to concentration increases of these chemicals and stimulating the detoxification pathways, resulting in a decreased interaction of EDCs with other NRs. This PXR-driven elimination of xenobiotics confers a positive role to the NR activation. On the contrary, PXR activation can also prevent the effects of endogenous hormones or drugs by premature stimulation of their metabolism leading to adverse interactions or harmful effects. In addition, PXR activation has been linked to chemoresistance, growth and aggressiveness of colon and hepatic cancers (Banerjee et al, 2014; Kodama et al, 2015; Wang et al 2011).

Unlike many NRs that specialize in binding of ligands with structural homologies, PXR is able to interact with large number of structurally diverse compounds with medium affinities (Kds between 0.1 and 100 micromolar). Known PXR ligands include pesticides, phenols, cosmetics, phytoestrogens, pharmaceuticals, etc (table 1) (Banerjee et al, 2014). Crystallographic studies have revealed the unique PXR characteristics accounting for its promiscuous ligand-binding properties. Firstly, PXR possesses a large LBP that can accommodate compounds with larger volumes than classical NR ligands, and secondly, several LBD loops confer a high plasticity allowing the receptor to adopt different shapes considering the bound ligands (le Maire et al, 2010). A recent study demonstrate that both pharmaceutical estrogen (contraceptive 17 α -ethinylestradiol, EE2) and persistent

organochlorine pesticide (*trans*-nonachlor, TNC), exhibit low efficacy if studied separately, but cooperative binding to PXR leads to synergistic activation (Delfosse et al, 2015b). High-resolution crystal structures showed that EE2 and TNC individually are too small to make all the necessary interactions ensuring high binding affinity and effective stabilization of the receptor active conformation. In contrast, when associated in a binary mixture, EE2 and TNC fill larger fraction of the PXR LBP. Strong inter-ligand contacts between EE2 and TNC generate a mutual stabilization of the compounds in the LBP and account for the enhanced binding affinity of the binary mixture. This study provided the first detailed mechanistic explanation as well as the proof of concept for the synergistic action of a mixture of compounds (cocktail effect) *via* their simultaneous interaction with NR. Similarly, other compounds among estrogens and pesticides were shown to bind and activate PXR at much lower concentration than individually (Delfosse et al, 2015b; our unpublished results).

The protective, detoxification system against external chemicals including environmental disruptors is also regulated by CAR. According to its expression in small intestine, CAR is also responsible for regulation of glucose/lipid metabolisms (elimination of cholesterol) and maintaining energy homeostasis under both, physiological and pathological conditions (Kobayashi et al, 2015). CAR was considered to be less promiscuous than PXR according to its lower LBD flexibility (Buchman et al, 2018; Wu et al, 2013). However, the evidences that CAR is also activated by numerous EDCs of variable structure like phthalates, alkylphenols, bisphenols and pesticides are mounting (Kamata et al, 2018; Kojima et al, 2019; Laurenzana et al, 2017; Liu et al, 2019; Lynch et al, 2019; Wahlang et al, 2014; Zhang et al, 2015 and our unpublished results).

3.4 Peroxisome proliferator activated receptors

The NR subfamily of PPARs includes three members, PPAR α (NR1C1), PPAR β/δ , and PPAR γ . These receptors bind to PPAR-responsive DNA regulatory elements in the form of heterodimers with RXR. PPARs have distinct tissue distributions and physiological roles (Michalik et al, 2006). PPAR α is preferentially expressed in the heart, liver, and brown adipose tissue, whereas PPAR β/δ is expressed ubiquitously. They both play an important role as activators of fatty acid oxidation pathways and thus in the regulation of energy homeostasis. PPAR γ , for its part, is highly expressed in adipose tissues and plays a key role in adipogenesis regulation, lipid metabolism, and glucose homeostasis by improving insulin sensitivity. PPARs bind and respond to dietary fatty acids and various lipid metabolites, including eicosanoids, prostaglandins, and oxidized phospholipids. In accordance with their tissue distributions and roles as sensors of lipids/fatty acids levels, in regulating fatty acid catabolism, and in lipid storage, all three PPARs are thought to be strongly involved in the metabolic syndrome.

In the light of particular PPAR γ role in adipose tissue development and maintenance, it has been suggested that the disruption of regulatory pathways may be specifically implicated in the onset of diabetes and obesity. Thus PPAR γ activation by EDCs could contribute to the “obesogen hypothesis” stating that the growing obesity epidemics due to the imbalance between caloric intake and expenditure could also implicate chemicals, so-called “obesogens,” which directly or indirectly increase fat accumulation and obesity. As a matter of fact, PPAR γ activation by some xenobiotic compounds like tetrabromobPA, perfluorooctanoic acid, mono(2-ethylhexyl) phthalate (MEHP) and organotins has been linked to *in vitro* and *in vivo* by stimulation of adipogenesis promoting by the differentiation of the fibroblastic lineage preadipocytes into mature adipocytes (Chappell et al, 2018; Grun et al 2014, Watt and Schlezinger, 2015; Zhang et al, 2014). With the exception of organotins that can bind to PPAR γ with nanomolar affinities, all of these compounds activate PPAR γ with EC₅₀s in the micromolar range (table 1). The crystal structure of PPAR γ in complex with the tripropyltin (Delfosse et al, 2015b) shows that the interacting cysteine anchors the organotin in the LBP region, so the efficient stabilization of the active receptor conformation is not allowed. This is in line with the weak PPAR γ agonistic activity of the compound.

Interestingly, among PPAR γ EDCs, PFOAs and MEHP are also able to activate PPAR α (Fang et al, 2015; Lapinskas et al, 2005; Wolf et al, 2014) suggesting that the effect of these compounds could be due to dual actions on both PPARs.

3.5 Thyroid Receptors

TRs fulfill essential roles in the normal development, growth and metabolism of all vertebrates and is involved in neurogenesis and brain functioning (Bernal, 2007; Zoeller et al., 2002). TR α is mainly expressed in the heart whereas TR β expression pattern is restricted to the liver and the brain and is developmentally regulated. These nuclear transcription factors respond to endogenous circulating thyroid hormones (THs) thyroxine (T₄) and its active form, 3,3',5-triiodothyronine (T₃) that are secreted by thyroid gland. TH signaling can be disturbed by chemicals affecting the thyroid gland synthesis, transport, metabolism and cellular uptake of circulating THs and interaction with TRs (Crofton, 2008 Ghassabian et al, 2018) resulting in numerous malfunctions emerging brain development, reproduction or blood circulation. Polybrominated diphenylethers (PBDEs), bromophenols, PFOAs, halogenated bisphenols as well as fipronil have been shown to cause hypothyroidism (Coperchini et al, 2017; Fini et al, 2012; Roques et al, 2012). They act as TRs antagonist with low potency.

3.6 Retinoic acid receptors

RARs are nuclear receptors that respond to all-*trans* retinoic acid (RA), metabolites of vitamin A. RA is essential for cellular proliferation, development and differentiation and thus

plays crucial roles in growth and homeostasis in vertebrates. Vitamin A deficiency in the developing embryo results in various organs malformations while RA excess causes teratogenesis (Lhones et al, 1995; Zile, 2001). RAR α is ubiquitously expressed whereas RAR β and RAR γ are more selective. RARs selective ligands have been reported to induce specific defects in mice (Elmazar, 2001). Interestingly, some studies have identified several chemicals among different classes of EDCs, chemicals that bind to and activate RARs in the micromolar range (Lemaire et al, 2005; Kamata et al, 2008; 2018).

3.7 Farnesoid X Receptor and liver X receptors

FXR and LXRs are nuclear receptors that function as intracellular sensors for sterols and bile acids respectively, maintaining a balanced, finely tuned regulation of their metabolism. FXR is abundantly expressed in liver, intestine, adipose tissue, adrenal glands and kidney (Preidis et al, 2017). FXR is endogenously activated by physiological concentrations of free and conjugated bile acids (Moore et al, 2006) and was involved in the pathogenesis of different cholestatic diseases, as well as non-alcoholic fatty liver disease and inflammatory bowel disease (Armstrong and Guo, 2017; Tanaka et al, 2017). Specifically, treatment with FXR agonists has shown beneficial roles in regulating glucose homeostasis and insulin sensitivity (Jiao et al, 2015). Activation of FXR also reduces the triglyceride level in liver tissues, suggesting the therapeutic effects of FXR agonists in non-alcoholic fatty liver disease (NAFLD) (Sepe et al, 2018; van de Wiel et al, 2019).

LXR α and LXR β are highly conserved among rodents and human. LXR α is highly expressed in the liver but is also found in kidney, intestine, adipose tissue, and macrophages, whereas LXR β is expressed ubiquitously. The endogenous activators of LXRs are oxysterol activating both LXR α and LXR β (Moore et al, 2006) as well as other derivatives of cholesterol metabolism. As such, they participate in the cholesterol sensing and regulate important aspects of cholesterol and fatty acid metabolism (Kirchgessner et al, 2016; Tontonoz and Mangelsdorf, 2003).

Mounting evidence suggests that LXRs and FXR could be important targets of EDCs. Some compounds have been already showed to bind to FXR and LXRs (Hiebl et al, 2018; Hsu et al, 2016). Furthermore, PXR, LXRs and FXR share common ligands among pharmaceuticals that suggest environmental PXR ligands could also interact with FXR and LXRs (Carazo et al, 2017; Whaland et al, 2014; Xue et al, 2007 and our unpublished results). Further investigation is needed to find out whether EDCs can negatively contribute to disease development (eg NAFLD).

4 Estrogen related receptor γ

ERRs are a family of three NRs based on their high level of homology with ERs. However, despite this particularity, ERRs do not bind estrogens and play key roles in control of cellular energy metabolism. Indeed, they are expressed in tissues with high energetic demands targeting transcriptional regulation of metabolic genes involving mitochondrial functions, lipid, carbohydrate, pyruvate, amino/nucleic acid metabolisms, as well as cellular energy metabolism (Audet-Walsh and Giguère, 2015). Any of three subunits ERR α , ERR β and ERR γ can recognize the same response elements and interfere with estrogen signaling (Giguere, 2002). ERR γ has been proposed to act as a mediator of low-dose effects of some environmental endocrine disrupting chemicals (EDCs) such as the xenoestrogen bisphenol-A (BPA) (Okada et al, 2008). However, other studies have shown that ERR γ activation by BPA occurs at concentrations about 100 nM (Abad et al 2008; Delfosse et al, 2012; Li et al, 2010; Thouenon et al, 2019). ERR γ also binds plenty of xenoestrogens, including several phenols and pesticides in the micromolar range (Thouenon et al, 2019).

5 Conclusion

EDCs represent a broad class of substances that are suspected to interfere with hormone synthesis, transport, action or degradation, thus altering the proper function of the endocrine system. Deregulation of NR-mediated transcription appears to account for the number of EDCs deleterious effects. Thus, the precise characterization of NRs-EDCs interaction is important for the assessment of the global hormonal activity of these chemicals as well as the understanding of their action mechanisms at environmentally relevant concentrations. The high-affinity interaction between some EDCs and NRs could explain some of these effects. For instance, in spite of its structural differences with E2, the interaction of α -ZA (a mycoestrogen with non-estrogenic chemical structure) with ER α resembles that of endogenous ligand, E2. Consequently, α -ZA and its derivatives are the most active xenoestrogens modulating ER activity at low nanomolar concentrations.

In contrast, it has been shown that organotins such as TBT do not resemble any of the classical RXRs and PPAR γ interactions. Instead, tin compounds covalently bind (Sn-S covalent interaction) and modulate the transcriptional activity of the RXR–PPAR heterodimer at nanomolar concentrations. Thus, the low dose effects of some EDCs could also be explained by their covalent interaction with the NRs containing a cysteine residue in their LBP. Accordingly, dibutyltin has been reported as the potent antagonist of GR that contains two cysteines residues in its hormone-binding site. Covalent coupling between NRs and pharmaceutical or natural compounds has also been reported (Egawa et al, 2016; Leesnitzer et al, 2002). Interestingly, we have observed that other EDCs can establish covalent bonds with reactive NRs cysteines (Balaguer et al, 2019).

The concomitant interaction of two EDCs with PXR in low concentrations has been recently

proposed as another potent mechanism of action. It is referring that two compounds mutually interact within PXR LBP, forming a more potent activator than the chemicals individually. These two compounds not only bind concomitantly to receptor LBP, but they work cooperatively, *i.e.* binding of one molecule promotes high affinity binding of the other. Structural studies revealed that ligands mutually stabilize each other *via* strong inter-ligand contacts and that a large number of interactions with LBP amino acids allow to “supramolecular ligand” tightly hold the receptor in the transcriptionally-competent conformation. The synergistic/additive effect resulting from the simultaneous activation or inhibition of different NRs could also account for the low dose action of certain chemicals. Cooperative co-binding of synthetic and natural ligands to PPAR γ has also been observed (Shang et al, 2018). Large PXR and PPARs LBPs are obviously predisposed to simultaneously accommodate several ligands. In contrast to the perception that the most NRs possess a well-defined pocket, several structural studies have revealed their LBPs exhibit a greater conformational flexibility than previously thought. Importantly, this structural adaptability allows ER α , TR β , GR and ERR γ to expand their binding pocket and accommodate much larger ligands (Borngraeber et al, 2003; Nettles et al, 2007; Suino-Powell et al, 2008; Togashi et al, 2005). Synergistic or additive effects resulting in simultaneous binding to different NRs could also contribute to low dose effects of certain EDCs. BPA (ERs, ERR γ , PXR and CAR agonist and AR antagonist), MEHP and PFOAs (PPAR α and PPAR γ agonists) are good examples of EDC whose adverse effects could result from their combined action on several NRs. Finally, EDCs could also interact with associated receptors associated to the membrane. A fraction of NRs, including ER α and β , AR and G protein-coupled estrogen receptor (GPER) are located at the membrane and could mediate the effects of EDCs such as bisphenols and alkylphenols outside the nucleus. It has been suggested that such rapid nongenomic activation of signal transduction pathways could explain the effects of EDCs, but this hypothesis needs further investigations.

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Figure Captions

Figure 1. The main target nuclear receptor targets for endocrine disruptors and their natural (bold) and environmental ligands. * 9-*cis* RA is debated as natural ligand of RXRs.

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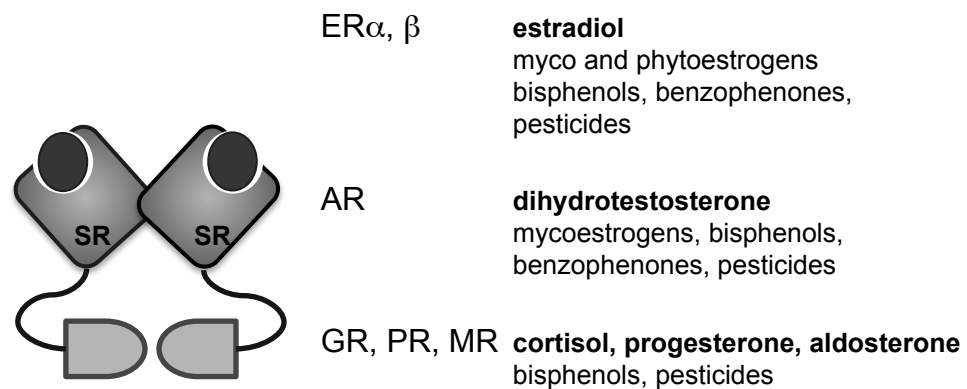
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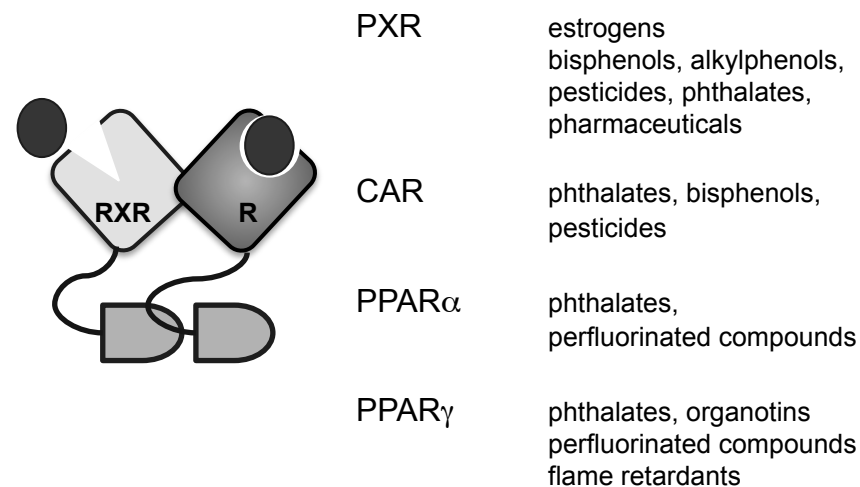
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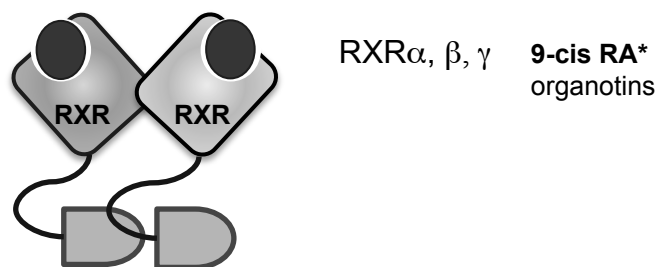
Steroid receptors



RXR partners



Dimeric Orphan Receptors



Monomeric/Tethered Orphan Receptors

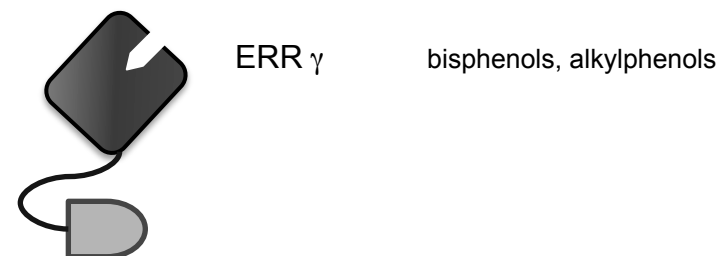


Fig 1

Table 1. EDCs and their NR targets

EDCs	NR targets	affinity	environmental compounds	nature
Natural estrogens	ER α , ER β PXR	high low	E2 E2	agonist agonist
Alkylphenols	ER α , ER β AR PXR CAR RAR γ ERR γ	medium low low low low medium	4-tert-Octylphenol 4-tert-Octylphenol 4-tert-Octylphenol Nonylphenol mixture 4-cumyl-phenol 4-cumyl-phenol	agonist antagonist agonist agonist agonist agonist
Benzophenones	ER α , ER β AR	medium low	BP2, THB BP2, THB	agonist antagonist
Bisphenols	ER α , ER β AR GR PXR CAR TRs ERR γ	medium medium low low low controversed medium	BPA, BPAF, BPC BPC BPP BPM BPZ BPA	agonist antagonist antagonist agonist antagonist agonist
Halogenated bisphenols	ER α , ER β PPAR γ TRs	low low low	TCBPA TBBPA, TCBPA TBBPA	agonist agonist antagonist
Mycoestrogens	ER α , ER β AR PXR	high low low	α -zearalanol agonist α -zearalanol α -zearalanol	agonist antagonist agonist
Organotins	PPAR γ RXR α GR	high high High	TPropT TBT DBT	agonist agonist antagonist
Parabens	ER α , ER β	low	butyl paraben	agonist
Perfluorinated compounds	PPAR α , PPAR γ	low	PFOA	agonist
Pesticides	ER α , ER β AR PXR CAR RAR γ ERR γ	low medium low low low medium	DDE, HPTE, M2 M2 vinclozolin, DDE pretilachlor, TNC 2,4'DDE, methoxychlore endrin HPTE	agonist agonist agonist agonist agonist agonist
Phytoestrogens	ER α , ER β PPAR γ	medium low	genistein, ferutinin genistein	agonist agonist
Phthalates	ER α , ER β PPAR γ CAR	low low low	BBP MEHP DHEP	agonist agonist agonist

BBP, benzyl butyl phthalate; BP2, benzophenone 2; BPA, bisphenol A; BPAF, Bisphenol AF, BPC, Bisphenol C; BPM Bisphenol M; BPP; Bisphenol P; BPZ, Bisphenol Z; DDE, dichlorodiphenyldichloroethylene; DEHP, diethylhexyl phthalate; E2, estradiol; EE2, ethynil estradiol; HPTE, 1,1,1-trichloro-2,2-bis(4-hydroxyphenyl)ethane; MEHP, mono ethyl hexyl phthalate; PFOA, perfluorinated octanoic acid; TBT, tributyltin; THB, trihydroxy-benzophenone; TNC trans-nonachlore; TpropT, tripropyltin. Kds of high affinity are lower than 1 nM. Kds of medium affinity range from 1 nM and 1 μ M. Kds of low affinity are higher than 1 μ M.