

MINIREVIEW

New Modes of Action for Endocrine-Disrupting Chemicals

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Endocrine-disrupting chemicals (EDC) are commonly considered to be compounds that mimic or block the transcriptional activation elicited by naturally circulating steroid hormones by binding to steroid hormone receptors. For example, the Food Quality Protection Act of 1996 defines EDC as those, that “may have an effect in humans that is similar to an effect produced by a naturally occurring estrogen, or other such endocrine effect as the Administrator may designate.” The definition of EDC was later expanded to include those that act on the estrogen, androgen, and thyroid hormone receptors. In this minireview, we discuss new avenues through which xenobiotic chemicals influence these and other hormone-dependent signaling pathways. EDC can increase or block the metabolism of naturally occurring steroid hor-

mones and other xenobiotic chemicals by activating or antagonizing nuclear hormone receptors. EDC affect the transcriptional activity of nuclear receptors by modulating proteasome-mediated degradation of nuclear receptors and their coregulators. Xenobiotics and environmental contaminants can act as hormone sensitizers by inhibiting histone deacetylase activity and stimulating mitogen-activated protein kinase activity. Some endocrine disrupters can have genome-wide effects on DNA methylation status. Others can modulate lipid metabolism and adipogenesis, perhaps contributing to the current epidemic of obesity. Additional elucidation of these new modes of endocrine disruption will be key in understanding the nature of xenobiotic effects on the endocrine system. (*Molecular Endocrinology* 20: 475–482, 2006)

THE CONCEPT OF endocrine disruption, the inappropriate modulation of the endocrine system by dietary and environmental chemicals, as a mode of action for xenobiotic chemicals in animals first burst into prominence with the publication of *Our Stolen Future* (1). Since then, the topic has generated considerable controversy. Much of this controversy centers on determining what chemicals cause detectable adverse effects at exposure levels typically experienced by humans or animals. Experts disagree about which levels of exposure result in observable effects in animal studies. The issue remains unresolved and the area ripe for future investigation, because credible studies show the presence and absence of low-dose effects with the same chemicals and experimental models (2). However, it should be noted

that the existence of low dose effects is becoming more widely accepted. There is also disagreement about the degree of risk from exposure to endocrine-disrupting chemicals (EDC). Such risk was estimated to range from catastrophic (1) to unproven (3) to insignificant (4, 5). Also confounding the debate is the often vague definition of what constitutes an EDC. We will follow the standard espoused by Pickering and Sumpter (6) that the term endocrine disrupter should be reserved for chemicals whose primary effect is on the endocrine system via effects on receptor-mediated hormone action, hormone synthesis, or clearance.

Although EDC could influence the activity of peptide hormones as well as steroid hormones, this minireview will discuss only the effects of EDC on the actions of members of the nuclear receptor superfamily. We will focus on new and underappreciated mechanisms through which EDC might act. We will not consider the effect of dose, because, for the most part, these mechanisms are newly described, and appropriate animal studies remain to be performed. Considering that compounds exist (such as bisphenol A) that have been shown to be very weak estrogens using receptor activation and ligand binding studies, but potent estrogens in animal studies (7), we believe that a simplistic classification of EDC as strong or weak based solely on *in vitro* studies would be misleading and counterproductive.

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Abbreviations: AR, Androgen receptor; CAR, constitutive androstane receptor; DDT, 1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane; EDC, endocrine-disrupting chemical; EGME, ethylene glycol monomethyl ether; ER, estrogen receptor; MAA, methoxyacetic acid; PCB, polychlorinated biphenyl; PPAR γ , peroxisomal proliferator-activated receptor γ ; RXR, retinoic acid X receptor; SXR/PXR, human steroid and xenobiotic receptor/rodent pregnane X receptor; TBT, tributyltin.

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ENDOCRINE DISRUPTION BY MODULATING STEROID HORMONE METABOLISM

Steroid hormones are a large class of lipophilic molecules that act on a variety of target sites to regulate many physiological functions. Sexual and reproductive development is closely regulated by androgens, estrogens, and progestins. Inappropriate activation or antagonism of the sex steroid receptors is the most extensively studied model for endocrine disruption, particularly interference with estrogen receptor signaling, and will not be reviewed here. Instead, we will consider other receptor-mediated mechanisms that alter the bioavailability of endogenous steroid hormones.

Increasing or decreasing steroid metabolism could contribute to the detrimental effects of EDC. Two nuclear receptors, human steroid and xenobiotic receptor/rodent pregnane X receptor (SXR/PXR) (8, 9) and constitutive androstane receptor (CAR) (10, 11), are important regulators of xenobiotic and steroid hormone metabolism; therefore, their potential roles in endocrine disruption bear closer examination. SXR/PXR and CAR are highly expressed in the liver and intestine, where they mediate the induction of cytochrome P450 enzymes (e.g. CYP3A, CYP2B, and CYP2C (12), conjugation enzymes (e.g. UGT1A1) (13), and transporters (e.g. P-glycoprotein, multidrug resistance-associated proteins, and organic anion transporter peptide 2) (14) in response to xenobiotic ligands and steroid hormones. SXR/PXR and CAR regulate overlapping sets of target genes involved in xenobiotic metabolism (e.g. CYP3A and CYP2B) and also function in the regulation of bile acid synthesis and cholesterol metabolism (15). SXR, like most nuclear receptors, activates transcription upon ligand binding. In contrast, CAR is constitutively active under most circumstances, and its high basal activity is repressed by steroids related to androstenol (10) as well as by unliganded SXR (16).

Several classic endocrine-disrupting compounds alter CAR activity and the expression of its target genes. *Trans*-nonachlor, a component of the banned pesticide chlordane, repressed the basal activity of mouse CAR (17). The persistent environmental contaminant 1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethylene, itself a metabolite of the banned pesticide 1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane (DDT), increased the transcriptional activity of both rat CAR and rat PXR (18). Methoxychlor is a structural analog of and substitute for DDT that has a relatively short half-life in the environment and in animals and appears to be less toxic in mammals (19). However, methoxychlor can activate CAR and SXR/PXR (20–23) (Tabb, M. M., and B. Blumberg, unpublished observations). Sexual and reproductive abnormalities observed in male rats exposed to DDE have been attributed to endocrine disruption via binding to the androgen receptor (AR) (24, 25), because the related compound methoxychlor and its

metabolites are both antiandrogenic (26, 27). These compounds also activate CAR and/or SXR/PXR (see below), presenting another possible mechanism for their observed endocrine-disrupting effects.

SXR/PXR is unusual among nuclear receptors in that it has a broad ligand specificity and is activated by a large number of EDC. The organochlorine pesticides, di-(2-ethylhexyl) phthalate and nonylphenol, were found to be mouse PXR activators and CYP3A inducers (28, 29). Bisphenol A, an estrogenic compound used in the manufacture of plastics, activates human SXR (30). A more extensive analysis of 54 xenobiotics of environmental concern found that alachlor, benzophenone, benzene hexachloride, methoxychlor, nonylphenol, trifluralin, and vinclozolin activated rat PXR and induced the expression of CYP3A (23). Many EDC that activate SXR/PXR were previously reported to have developmentally toxic, estrogenic, and/or antiandrogenic effects (31–35). Activation of SXR/PXR and CAR and up-regulation of their target genes by the many compounds mentioned above can increase the levels of endocrine-disrupting metabolites while at the same time altering the local bioavailability of endogenous androgens and estrogens. This provides a route through which EDC can alter steroid receptor activity without directly binding to steroid receptors.

SPECIES-SPECIFIC EFFECTS

Certain xenobiotic compounds exhibit species-specific effects on SXR/PXR activation and target gene induction. A particularly interesting group of such compounds is the polychlorinated biphenyls (PCBs), a family of ubiquitous, persistent, bioaccumulated environmental contaminants. PCB exposure was linked to adverse effects in animals and wildlife, which ultimately led to a worldwide ban on their production and use. It has been difficult to reconcile the effects observed with individual PCBs, because populations are typically exposed to complex mixtures rather than a single congener. Some PCBs display classical endocrine-disrupting effects in their ability to bind to the estrogen receptor (ER) (36), to inhibit estrogen catabolism (37), or to interfere with normal signaling through the thyroid hormone receptor (38) and androgen receptor (39). In addition to their effects on endocrine receptors, some PCBs were able to activate mouse PXR (28). We explored the relationship between PCB structure and SXR/PXR activation and showed that although highly chlorinated PCBs activated rodent PXR, the same compounds bound to and antagonized human SXR, inhibiting the expression of genes involved in three phases of hormone and xenobiotic metabolism (40). To our knowledge, this is the first example of a ligand acting as an agonist on a particular nuclear receptor in one species, but as an antagonist on the orthologous receptor in a different species. Because rats are the primary pharmacological and

toxicological model organism, the obvious inference is that the use of data generated in rats to predict the risk of human exposure to these PCBs or mixtures that contain them will probably lead to erroneous conclusions. The ability of xenobiotics, such as these PCBs, to block activation of SXR/PXR illustrates another possible avenue of endocrine disruption: interference with the metabolism of naturally occurring steroid hormones, bioactive dietary compounds, and xenobiotics normally mediated by SXR/PXR.

ENDOCRINE DISRUPTION BY MODULATING NUCLEAR RECEPTOR COACTIVATORS

Nuclear receptors activate transcription by binding directly to hormone response elements in the regulatory region of target genes, recruiting a suite of coactivator proteins and the basal transcription machinery. Coactivators include the p160 family [steroid receptor coactivator-1 (SRC-1), transcriptional intermediary factor 2 (TIF2)/glucocorticoid receptor interacting protein 1 (GRIP1), and activator of thyroid and retinoic acid receptor (ACTR)/amplified in breast cancer 1 (AIB-1)/p300/CBP-associated factor (PCAF)] (41, 42), which have intrinsic histone acetyl transferase activity, and the thyroid hormone receptor activator protein 220/vitamin D receptor-interacting protein 205/peroxisomal proliferator-activated receptor (PPAR- γ)-binding protein, which lacks intrinsic histone acetyl transferase activity (43). Tissue-specific differences in coactivator levels regulate nuclear receptor activation, as does general competition for coactivators among nuclear receptors and other transcription factors.

Alterations in the expression levels of receptor and/or coregulator mRNAs and proteins would be expected to modulate receptor activity. In one example, drug treatment has been shown to increase steady-state nuclear receptor coactivator levels, thereby increasing transcriptional activation of ER α in the presence of xenobiotics (44). Similarly, the EDC bisphenol A increased expression levels of the coactivator thyroid hormone receptor activator protein 220 and increased expression of ER β in mouse uterus. The effects were different in Ishikawa endometrial cells, where bisphenol A only increased the expression of ER β (45). This result is similar to observations with selective ER modulators, which can increase steady-state nuclear receptor coactivator levels, thereby increasing transcriptional activation of ER α (44), but differs in that bisphenol A was also able to increase mRNA and protein levels of the ER β receptor itself in some cell types. These findings imply that an EDC can modulate target gene expression by altering coregulator and transcription factor levels, and that this modulation may be tissue specific. This is a possible new mechanism of action for a subset of xenobiotics.

A more subtle type of endocrine disruption can result from competition between steroid receptors and

xenobiotic receptors for transcriptional coactivators. For example, CAR can inhibit ER-mediated transcriptional activity without binding to an estrogen response element (46). CAR overexpression led to a dose-dependent reduction of ER activity. This effect was potentiated by further activating CAR with 1,4-bis[2-(3,5-dichloropyridyloxy)]benzene, whereas the CAR antagonist, androstenol, relieved the CAR-mediated repression of ER transcriptional activity. CAR repression of ER was relieved by increasing amounts of the coactivator GRIP-1 (46). This suggests that many xenobiotic activators of CAR, and by implication SXR/PXR, may have endocrine-disrupting effects on steroid hormone receptors by limiting coactivator availability.

EDC EFFECTS ON THE PROTEASOME-MEDIATED DEGRADATION OF NUCLEAR RECEPTORS

Several members of the nuclear receptor superfamily are known to be degraded through the ubiquitin-proteasome pathway in a ligand-dependent manner. Receptor turnover by proteasome-targeted degradation prevents cells from overstimulation by endogenous hormones or other activating signals and may also reset the transcriptional apparatus in preparation for a subsequent response (47). Inhibition of the ubiquitin-proteasome degradation pathway down-regulates the transcriptional activity of nuclear steroid receptors such as progesterone receptor (48) and AR (49, 50). ER α undergoes different rates of proteasome-mediated degradation in the presence of ER agonists, antagonists, and selective ER modulators, demonstrating that transcriptional activity can be affected by modulating receptor stability (51). This leads to the hypothesis that EDCs could act on proteasome-mediated degradation of nuclear receptors or coregulatory proteins to directly affect the magnitude and duration of normal hormonal responses, thereby causing endocrine-disrupting effects.

Masuyama and colleagues (52) compared the effects of bisphenol A and estradiol treatments on ER-mediated transcription. Both ER α and ER β interacted directly with SUG1 (suppressor for Gal 1), a component of the proteasome, in the presence of estradiol. In contrast, bisphenol A activated ER-mediated transcription, but did not enhance the interaction between ER β and SUG1. ER β degradation was also much slower in the presence of bisphenol A than in the presence of estradiol or another estrogenic EDC, phthalic acid (52). Inhibition of ER β degradation should increase ER β protein levels, potentiating ER β transcriptional activation by bisphenol A and increasing its endocrine-disrupting effects. This could explain previous observations relating to differential effects of bisphenol A treatment on ER levels (45). Bisphenol A is currently controversial, with a number of academic

studies demonstrating *in vivo* effects at low levels, whereas others dispute the low-dose effects, noting that bisphenol A is a weak ER activator (53).

The transcriptional activity of other nuclear receptors, such as SXR/PXR, is also regulated by proteasome degradation. Phthalic acid was able to block the normal proteasome-mediated degradation of PXR compared with the endogenous PXR ligand, progesterone. This raises the possibility that endocrine disruptors, such as phthalic acid, may increase PXR protein levels and thereby alter the expression of PXR target genes (54). In turn, this could affect the clearance of endogenous hormones. Although one would intuitively expect the normal homeostatic mechanisms to compensate and maintain circulating steroid hormone levels, there is evidence that the induction of metabolic pathways by xenobiotics leads to increased circulating steroid hormone levels (55–57). Even small changes in the levels of circulating sex steroids during critical periods of development would be expected to have endocrine-disrupting effects. Other groups have also shown that p160 family coactivators, such as GRIP1 and SRC-1, are degraded via the proteasome (44, 58), potentially broadening this research avenue.

ENDOCRINE DISRUPTERS AS HORMONE SENSITIZERS

Recent work by Jansen and colleagues (59) has demonstrated a new mechanism of endocrine disruption. Their results show that xenobiotic short-chain fatty acids valproic acid and methoxyacetic acid (MAA) do not mimic endogenous hormones, but, rather, increase hormone receptor (ER α , ER β , AR, progesterone receptor, and thyroid hormone receptor β) activity by altering cell signals that activate protein kinases or inhibit histone deacetylases (59). Histone deacetylases normally remove an acetyl group from histones, which allows histones to bind DNA and inhibit gene transcription. Valproic acid is a commonly prescribed anticonvulsant and mood stabilizer (60, 61). Mechanistically, valproic acid acts as a histone deacetylase inhibitor while also increasing the expression of the cell cycle regulator p21 (62). Methoxyethanol, also known as ethylene glycol monomethyl ether (EGME), is a solvent commonly in paints, dyes, and fuel additives, and it has been known for some time that MAA, an EGME metabolite, can potentiate the effects of testosterone and increase the expression of ER β (63, 64).

Exposure to these short-chain fatty acids increased cellular sensitivity to estrogens, progestins, and other nuclear hormone receptor ligands and enhanced the transcriptional efficacy of ligand-activated nuclear hormone receptors *in vitro* and *in vivo* (59). Mechanistic studies revealed that these xenobiotics function as both activators of p42/p44 mitogen-activated protein kinase and inhibitors of histone deacetylases at doses

that parallel known human exposure levels. The likely target of increased activation of MAPK is the phosphorylation of coactivators (65), whereas the inhibition of histone deacetylase activity has more general effects on nuclear receptor activity.

These findings suggest that individuals who are exposed to these short-chain fatty acids are more likely to experience side effects from the administration of exogenous estrogens and progestins, including those given for oral contraception and postmenopausal hormone replacement therapy. An additional consideration would be the potentiation of endogenous steroid hormone effects. Therefore, these hormone sensitizers represent a new nuclear receptor interaction pathway relevant to endocrine disruption. A particular concern is the prevalence of human exposure to both valproic acid and MAA. Occupational exposure to EGME/MAA is widespread in the semiconductor and painting industries, whereas human exposure to valproic acid, such as Depakote (Abbott Laboratories, Abbott Park, IL), is widespread because it is among the top 200 prescription drugs dispensed in the United States (66).

EDCs LEAD TO TRANSGENERATIONAL EFFECTS ON FERTILITY BY REPROGRAMMING DNA METHYLATION IN THE MALE GERM LINE

DNA in primordial germ cells is demethylated and then remethylated in a sex-specific manner during gonadal sex determination (67), and DNA methylation controls gene expression (68). EDCs acting inappropriately through nuclear receptors such as AR and ER β during gonadal sex determination could reprogram the germ line by interfering with the fidelity of this process. Recently, two EDCs, methoxychlor and vinclozolin, were shown to alter the spermatogenic capacity of male germ cells and sperm viability via their effects on DNA methylation. The properties of methoxychlor as a replacement for the pesticide DDT were described above, and vinclozolin is a fungicide used in the wine industry that is actually metabolized into more active antiandrogenic compounds (32). A transient embryonic exposure to vinclozolin or methoxychlor during gonadal sex determination in the rat (embryonic d 8–15) reduced fertility and sperm development in the adult testis. Remarkably, this phenotype transmitted through the male germ line to at least the F4 generation with no additional exposure (69). Interestingly, the phenotype was observed in nearly all males from EDC-treated generations and was found to be associated with modulation of genome-wide DNA methylation patterns in the male germ line (69). Exposure levels in the rat studies were higher than a typical environmental exposure, but the epigenetic effects on male fertility caused by these EDCs points to an important new mechanism for EDC disruption of gene expression.

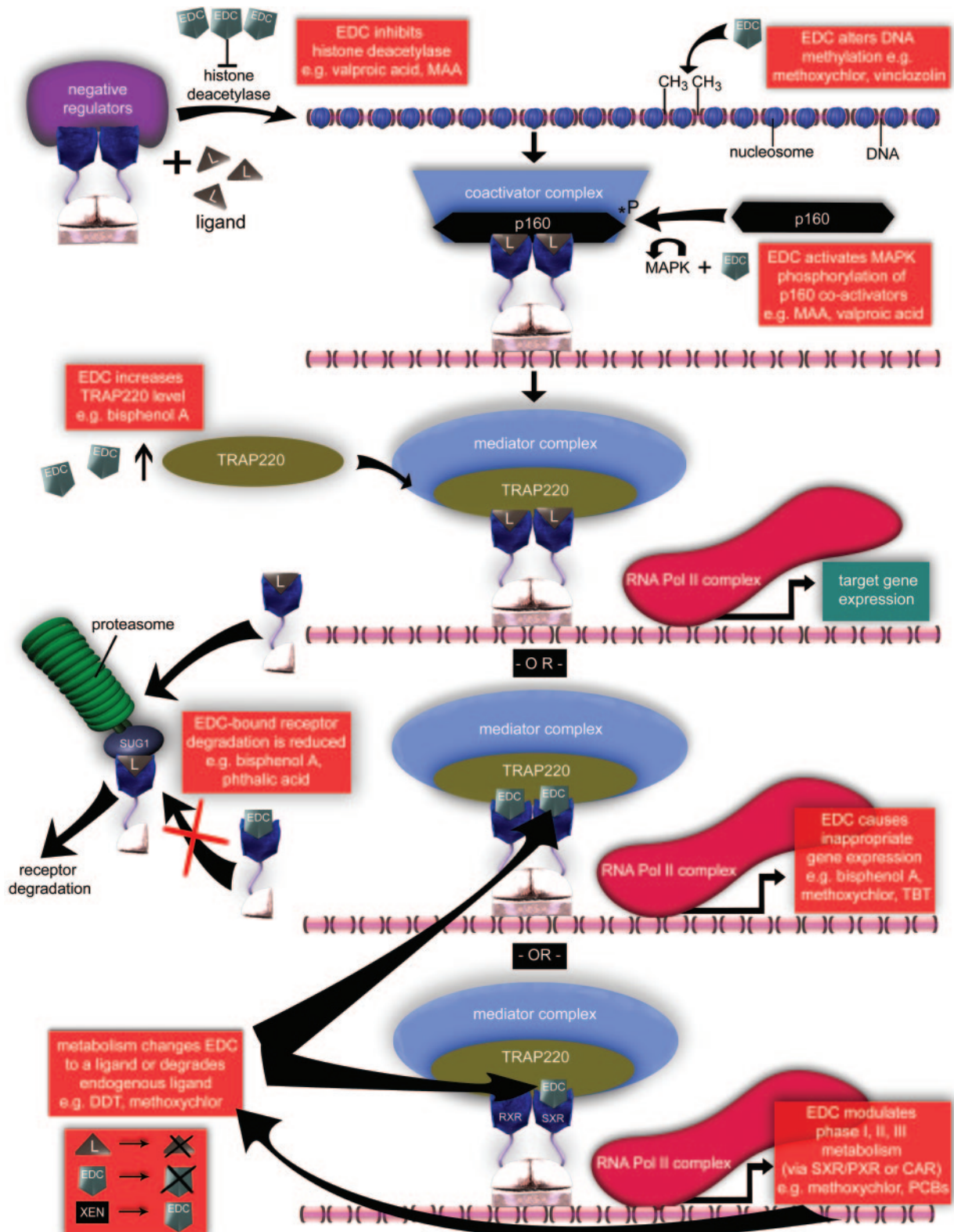


Fig. 1. Multiple Modes of Endocrine Disruption

Endocrine disruptors have effects on many aspects of transcription and transcriptional regulation that influence normal target gene expression. The red boxes indicate new modes of endocrine disruption focused on in this review. L, Ligand; XEN, xenobiotic.

ENDOCRINE DISRUPTERS AS OBESOGENS

A recent review summarized the potential role of EDC effects via ER on the growing obesity epidemic (70). However, other nuclear receptors are also playing roles in the EDC effects on obesity. We (Grün, F., H. Watanabe, Z. Zamanian, L. Maeda, K. Arima, R. Chubacha, D. M. Gardiner, T. Iguchi, J. Kanno, and B. Blumberg, unpublished results) and others (71) recently showed that tributyltin (TBT) could activate PPAR γ and retinoic acid X receptors (RXRs) at environmentally relevant (nanomolar) levels and that TBT treatment induced adipocyte differentiation in the 3T3-L1 adipogenesis model. TBT represents, to our knowledge, the first example of an environmental EDC that promotes adipogenesis through RXR and PPAR γ . Developmental or chronic lifetime exposure to TBT and other organotins could act as chemical stressors or obesogens that activate RXR and/or RXR:PPAR γ signaling to promote long-term changes in adipocyte number and/or lipid homeostasis. The effects of EDC on other nuclear receptors that modulate lipid metabolism, such as PPAR α , liver X receptor, and farnesoid X receptor, remain largely unexplored, making this a hot topic for future investigation.

CONCLUDING REMARKS

Endocrine disruption has previously been associated with inappropriate modulation of ER, AR, and thyroid hormone receptors. The examples of endocrine disruption discussed above underscore the complexity of ligand-activated nuclear receptor transcription and point to a large number of potential targets for xenobiotic disruption of endogenous hormone signaling (Fig. 1). Mechanisms involving the potential disruption of hormone metabolism, receptor protein degradation, sensitization by short-chain fatty acid exposure, altered DNA methylation, and effects on receptors other than ER, AR, and thyroid hormone receptor are underappreciated as potential routes for endocrine disruption. Genomic screens and molecular modeling have been used to identify endocrine disrupters that directly affect the activity of a few nuclear hormone receptors (72–75). The Food Quality Protection Act of 1996 required the EPA to develop a screening program to test chemicals and pesticides for potential endocrine-disrupting effects. To date, it has proven difficult to implement such a screening program, primarily due to the large number of different types of assays that would have to be employed to reliably identify and predict such effects *in vivo*. The growing number of potential modes of EDC action that do not directly affect ligand binding or receptor activation (and hence cannot be predicted by quantitative structure-activity relationship modeling or simple receptor activation assays) suggests that this task will only become more difficult in the future. Increased involve-

ment in the study of EDC action by biomedical scientists not normally working in this area, particularly those specializing in signaling, hormone action, and transcriptional regulation, will be key to our future understanding of endocrine disruption and its potential consequences for humans and wildlife.

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