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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 hormones 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; PPARγ, 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)/glucoccorticoid 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 ERα 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 ERβ in mouse uterus. The effects were different in Ishikawa endometrial cells, where bisphenol A only increased the expression of ERβ (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 ERα (44), but differs in that bisphenol A was also able to increase mRNA and protein levels of the ERβ 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). ERα 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 ERα and ERβ 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 ERβ and SUG1. ERβ 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 ERβ degradation should increase ERβ protein levels, potentiating ERβ 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 disrupters, 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 (ERα, ERβ, 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 ERβ (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 METHLYATION 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 ERβ 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 methoxylchor 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.

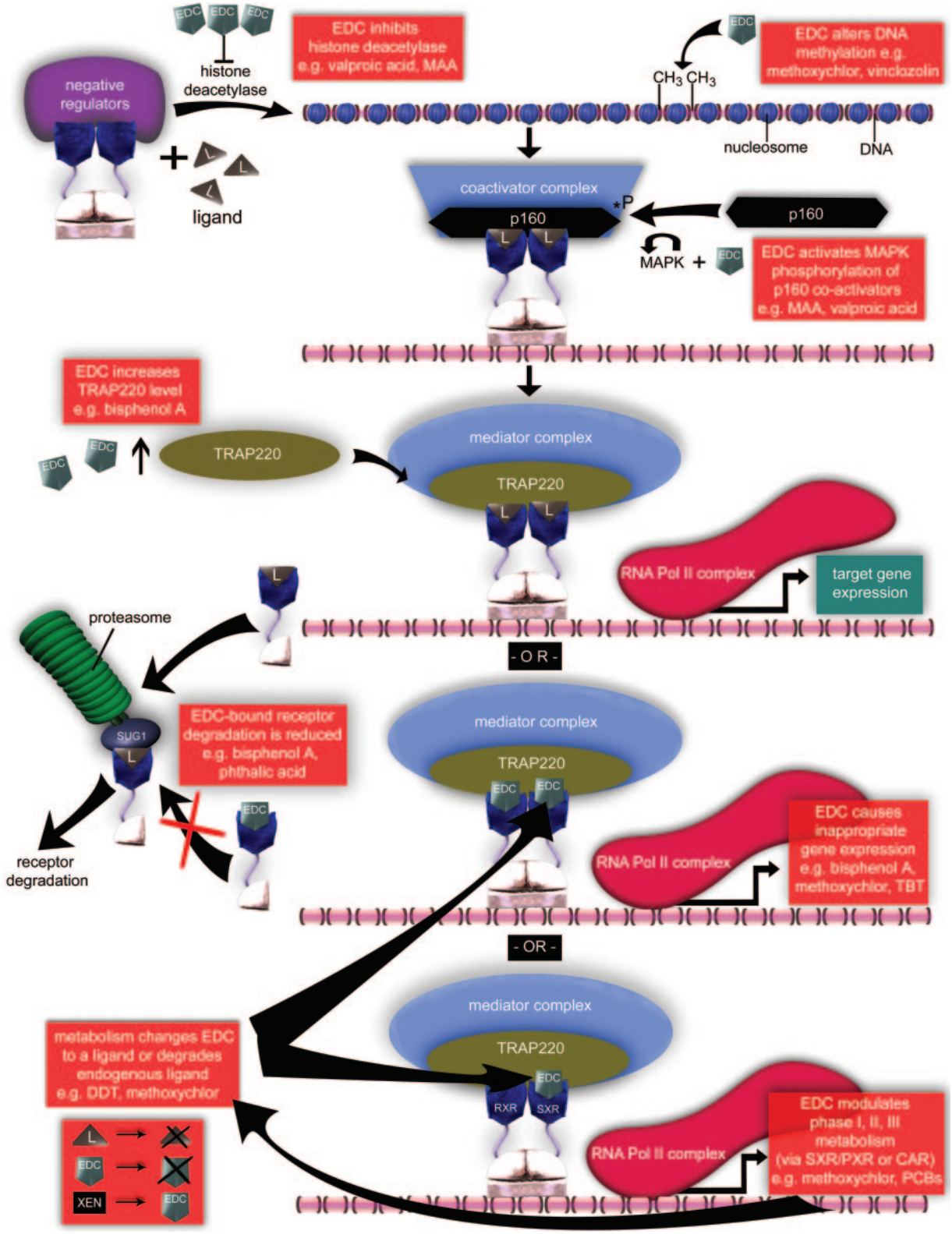

Fig. 1. Multiple Modes of Endocrine Disruption

Endocrine disrupters 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 PPARγ 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 PPARγ. Developmental or chronic lifetime exposure to TBT and other organotins could act as chemical stressors or obesogens that activate RXR and/or RXR:PPARγ 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 PPARα, 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 involvement 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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REFERENCES

1. Colborn T, Dumanoski D, Myers JP 1996 Our stolen future: are we threatening our fertility, intelligence and survival? New York: Penguin Grou
2. National Toxicology Program 2001 Endocrine disrupters low dose peer review. National Institute of Environmental Health Sciences, http://ntp.niehs.nih.gov/ntp/htdocs/liason/LowDosePeerFinalRpt.pdf
3. Safe SH 2000 Endocrine disruptors and human health–is there a problem? An update. Environ Health Perspect 108:487–493
4. Ames BN, Profet M, Gold LS 1990 Dietary pesticides (99.99% all natural). Proc Natl Acad Sci USA 87: 7777–7781
5. Ames BN, Gold LS 2000 Paracelsus to parascience: the environmental cancer distraction. Mutat Res 447:3–13
6. Pickering AD, Sumpter JP 2003 COMPREHENDing endocrine disrupters in aquatic environments. Environ Sci Technol 37:331A–336A
7. Welshons WV, Thayer KA, Judy BM, Taylor JA, Curran EM, vom Saal FS 2003 Large effects from small exposures. I. Mechanisms for endocrine-disrupting chemicals with estrogenic activity. Environ Health Perspect 111: 994–1006
8. Blumberg B, Sabbagh Jr W, Juguilon H, Bolado Jr J, van Meter CM, Ong ES, Evans RM 1998 SXR, a novel steroid and xenobiotic-sensing nuclear receptor. Genes Dev 12: 3195–3205
9. Kliewer SA, Moore JT, Wade L, Staudinger JL, Watson MA, Jones SA, McKee DD, Oliver BB, Wilson TM, Zetterstrom RH, Perlmann T, Lehmann JM 1998 An orphan nuclear receptor activated by pregnanes defines a novel steroid signaling pathway. Cell 92:73–82
10. Forman BM, Tzameli I, Choi HS, Chen J, Simha D, Seol W, Evans RM, Moore DD 1998 Androstane metabolites bind to and deactivate the nuclear receptor CAR-β. Nature 395:612–615
11. Xie W, Barwick JL, Simon CM, Pierce AM, Safe S, Blumberg B, Guzelian PS, Evans RM 2000 Reciprocal activation of xenobiotic response genes by nuclear receptors SXR/PXR and CAR. Genes Dev 14:3014–3023
12. Pascussi JM, Gerbal-Chaloin S, Drocourt L, Maurel P, Vilarem MJ 2003 The expression of CYP2B6, CYP2C9

and CYP3A4 genes: a tangle of networks of nuclear and steroid receptors. Biochim Biophys Acta 1619:243–253
13. Xie W, Yeuh MF, Radominska-Pandya A, Saini SP, Negishi Y, Bottroff BS, Cabrera GY, Tukey RH, Evans RM 2003 Control of steroid, heme, and carcinogen metabolism by nuclear pregnane X receptor and constitutive androstane receptor. Proc Natl Acad Sci USA 100: 4150–4155
14. Staudinger JL, Madan A, Carol KM, Parkinson A 2003 Regulation of drug transporter gene expression by nuclear receptors. Drug Metab Dispos 31:523–527
15. Guo GL, Lambert G, Negishi M, Ward JM, Brewer Jr HB, Kliewer SA, Gonzalez FJ, Sinal CJ 2003 Complementary roles of farnesoid X receptor, pregnane X receptor, and constitutive androstane receptor in protection against bile acid toxicity. J Biol Chem 278:45062–45071
16. Saini SP, Mu Y, Gong H, Toma D, Uppal H, Ren S, Li S, Poloyac SM, Xie W 2005 Dual role of orphan nuclear receptor pregnane X receptor in bilirubin detoxification in mice. Hepatology 41:497–505
17. Moore LB, Maglich JM, McKee DD, Wisely B, Willson TM, Kliewer SA, Lambert MH, Moore JT 2002 Pregnane X receptor (PXR), constitutive androstane receptor (CAR), and benzoate X receptor (BXR) define three pharmacologically distinct classes of nuclear receptors. Mol Endocrinol 16:977–986
18. Wyde ME, Bartolucci E, Ueda A, Zhang H, Yan B, Negishi M, You L 2003 The environmental pollutant 1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethylene induces rat hepatic cytochrome P450 2B and 3A expression through the constitutive androstane receptor and pregnane X receptor. Mol Pharmacol 64:474–481
19. Bulger WH, Kupfer D 1983 Effect of xenobiotic estrogens and structurally related compounds on 2-hydroxylation of estradiol and on other monooxygenase activities in rat liver. Biochem Pharmacol 32:1005–1010
20. Li HC, Kupfer D 1998 Mechanism of induction of rat hepatic CYP2B and 3A by the pesticide methoxychlor. J Biochem Mol Toxicol 12:315–323
21. Sueyoshi T, Kawamoto T, Zelko I, Honkakoski P, Negishi M 1999 The repressed nuclear receptor CAR responds to phenobarbital in activating the human CYP2B6 gene. J Biol Chem 274:6043–6046
22. Blizard D, Sueyoshi T, Negishi M, Dehal SS, Kupfer D 2001 Mechanism of induction of cytochrome p450 enzymes by the proestrogenic endocrine disruptor pesticide-methoxychlor: interactions of methoxychlor metabolites with the constitutive androstane receptor system. Drug Metab Dispos 29:781–785
23. Mikamo E, Harada S, Nishikawa J, Nishihara T 2003 Endocrine disruptors induce cytochrome P450 by affecting transcriptional regulation via pregnane X receptor. Toxicol Appl Pharmacol 193:66–72
24. Kelce WR, Stone CR, Laws SC, Gray LE, Kemppainen JA, Wilson EM 1995 Persistent DDT metabolite *p,p′*-DDE is a potent androgen receptor antagonist. Nature 375: 581–585
25. You L, Casanova M, Archibeque-Engle S, Sar M, Fan LQ, Heck HA 1998 Impaired male sexual development in perinatal Sprague-Dawley and Long-Evans hooded rats exposed in utero and lactationally to *p,p′*-DDE. Toxicol Sci 45:162–173
26. Ousterhout J, Struck RF, Nelson JA 1981 Estrogenic activities on methoxychlor metabolites. Biochem Pharmacol 30:2869–2871
27. Gaido KW, Maness SC, McDonnell DP, Dehal SS, Kupfer D, Safe S 2000 Interaction of methoxychlor and related compounds with estrogen receptor α and β, and androgen receptor: structure-activity studies. Mol Pharmacol 58:852–858
28. Schuetz EG, Brimer C, Schuetz JD 1998 Environmental xenobiotics and the antihormones cyproterone acetate and spironolactone use the nuclear hormone pregnenolone X receptor to activate the CYP3A23 hormone response element. Mol Pharmacol 54:1113–1117
29. Masuyama H, Hiramatsu Y, Kunitomi M, Kudo T, MacDonald PN 2000 Endocrine disrupting chemicals, phthalic acid and nonylphenol, activate pregnane X receptor-mediated transcription. Mol Endocrinol 14: 421–428
30. Takeshita A, Koibuchi N, Oka J, Taguchi M, Shishiba Y, Ozawa Y 2001 Bisphenol-A, an environmental estrogen, activates the human orphan nuclear receptor, steroid and xenobiotic receptor-mediated transcription. Eur J Endocrinol 145:513–517
31. Klotz DM, Beckman BS, Hill SM, McLachlan JA, Walters MR, Arnold SF 1996 Identification of environmental chemicals with estrogenic activity using a combination of in vitro assays. Environ Health Perspect 104:1084–1089
32. Kelce WR, Monosson E, Gamcsik MP, Laws SC, Gray Jr LE 1994 Environmental hormone disruptors: evidence that vinclozolin developmental toxicity is mediated by antiandrogenic metabolites. Toxicol Appl Pharmacol 126:276–285
33. Byrd RA, Markham JK, Emmerson JL 1995 Developmental toxicity of dinitroaniline herbicides in rats and rabbits. I. Trifluralin. Fund Appl Toxicol 26:181–190
34. Schlumpf M, Schmid P, Durrer S, Conscience M, Maerkel K, Henseler M, Gruetter M, Herzog I, Reolon S, Ceccatelli R, Faass O, Stutz E, Jarry H, Wuttke W, Lichtensteiger W 2004 Endocrine activity and developmental toxicity of cosmetic UV filters-an update. Toxicology 205: 113–122
35. Oropeza-Hernandez LF, Sierra-Santoyo A, Cebrian ME, Manno M, Albores A 2001 Ovariectomy modulates the response of some cytochrome P450 isozymes to lindane in the rat. Toxicol Lett 124:91–99
36. Bonefeld-Jorgensen EC, Andersen HR, Rasmussen TH, Vinggaard AM 2001 Effect of highly bioaccumulated polychlorinated biphenyl congeners on estrogen and androgen receptor activity. Toxicology 158:141–153
37. Kester MH, Bulduk S, Tibboel D, Meinl W, Glatt H, Falany CN, Coughtrie MW, Bergman A, Safe SH, Kuiper GG, Schuur AG, Brouwer A, Visser TJ 2000 Potent inhibition of estrogen sulfotransferase by hydroxylated PCB metabolites: a novel pathway explaining the estrogenic activity of PCBs. Endocrinology 141:1897–1900
38. Iwasaki T, Miyazaki W, Takeshita A, Kuroda Y, Koibuchi N 2002 Polychlorinated biphenyls suppress thyroid hormone-induced transactivation. Biochem Biophys Res Commun 299:384–388
39. Portigal CL, Cowell SP, Fedoruk MN, Butler CM, Rennie PS, Nelson CC 2002 Polychlorinated biphenyls interfere with androgen-induced transcriptional activation and hormone binding. Toxicol Appl Pharmacol 179:185–194
40. Tabb MM, Kholodovych V, Grun F, Zhou C, Welsh WJ, Blumberg B 2004 Highly chlorinated PCBs inhibit the human xenobiotic response mediated by the steroid and xenobiotic receptor (SXR). Environ Health Perspect 112: 163–169
41. Freedman LP 1999 Increasing the complexity of coactivation in nuclear receptor signaling. Cell 97:5–8
42. McKenna NJ, Lanz RB, O'Malley BW 1999 Nuclear receptor coregulators: cellular and molecular biology. Endocr Rev 20:321–344
43. Rachez C, Gamble M, Chang CP, Atkins GB, Lazar MA, Freedman LP 2000 The DRIP complex and SRC-1/p160 coactivators share similar nuclear receptor binding determinants but constitute functionally distinct complexes. Mol Cell Biol 20:2718–2726
44. Lonard DM, Tsai SY, O'Malley BW 2004 Selective estrogen receptor modulators 4-hydroxytamoxifen and raloxifene impact the stability and function of SRC-1 and SRC-3 coactivator proteins. Mol Cell Biol 24:14–24
45. Inoshita H, Masuyama H, Hiramatsu Y 2003 The different effects of endocrine-disrupting chemicals on estrogen

receptor-mediated transcription through interaction with coactivator TRAP220 in uterine tissue. J Mol Endocrinol 31:551–561
46. Min G, Kim H, Bae Y, Petz L, Kemper JK 2002 Inhibitory cross-talk between estrogen receptor (ER) and constitutively activated androstane receptor (CAR). CAR inhibits ER-mediated signaling pathway by squelching p160 coactivators. J Biol Chem 277:34626–34633
47. Dennis AP, Haq RU, Nawaz Z 2001 Importance of the regulation of nuclear receptor degradation. Front Biosci 6:D954–D959
48. Syvala H, Vienonen A, Zhuang YH, Kivineva M, Ylikomi T, Tuohimaa P 1998 Evidence for enhanced ubiquitin-mediated proteolysis of the chicken progesterone receptor by progesterone. Life Sci 63:1505–1512
49. Sheflin L, Keegan B, Zhang W, Spaulding SW 2000 Inhibiting proteasomes in human HepG2 and LNCaP cells increases endogenous androgen receptor levels. Biochem Biophys Res Commun 276:144–150
50. Lin HK, Altuwaijri S, Lin WJ, Kan PY, Collins LL, Chang C 2002 Proteasome activity is required for androgen receptor transcriptional activity via regulation of androgen receptor nuclear translocation and interaction with coregulators in prostate cancer cells. J Biol Chem 277: 36570–36576
51. Wijayaratne AL, McDonnell DP 2001 The human estrogen receptor-α is a ubiquitinated protein whose stability is affected differentially by agonists, antagonists, and selective estrogen receptor modulators. J Biol Chem 276:35684–35692
52. Masuyama H, Hiramatsu Y 2004 Involvement of suppressor for Gal 1 in the ubiquitin/proteasome-mediated degradation of estrogen receptors. J Biol Chem 279: 12020–12026
53. vom Saal FS, Hughes C 2005 An extensive new literature concerning low-dose effects of bisphenol A shows the need for a new risk assessment. Environ Health Perspect 113:926–933
54. Masuyama H, Inoshita H, Hiramatsu Y, Kudo T 2002 Ligands have various potential effects on the degradation of pregnane X receptor by proteasome. Endocrinology 143:55–61
55. Edwards OM, Courtenay-Evans RJ, Galley JM, Hunter J, Tait AD 1974 Changes in cortisol metabolism following rifampicin therapy. Lancet 2:548–551
56. Brodie MJ, Boobis AR, Gill M, Mashiter K 1981 Does rifampicin increase serum levels of testosterone and oestradiol by inducing sex hormone binding globulin capacity? Br J Clin Pharmacol 12:431–433
57. Lonning PE, Bakke P, Thorsen T, Olsen B, Gulsvik A 1989 Plasma levels of estradiol, estrone, estrone sulfate and sex hormone binding globulin in patients receiving rifampicin. J Steroid Biochem 33:631–635
58. Hoang T, Fenne IS, Cook C, Borud B, Bakke M, Lien EA, Mellgren G2004 cAMP-dependent protein kinase regulates ubiquitin-proteasome mediated degradation and subcellular localization of the nuclear receptor coactivator GRIP1. J Biol Chem 279:49120–49130
59. Jansen MS, Nagel SC, Miranda PJ, Lobenhofer EK, Afshari CA, McDonnell DP 2004 Short-chain fatty acids enhance nuclear receptor activity through mitogen-activated protein kinase activation and histone deacetylase inhibition. Proc Natl Acad Sci USA 101:7199–7204
60. Lammer EJ, Sever LE, Oakley Jr GP 1987 Teratogen update: valproic acid. Teratology 35:465–473
61. Dansky LV, Finnell RH 1991 Parental epilepsy, anticonvulsant drugs, and reproductive outcome: epidemiologic and experimental findings spanning three decades. II. Human studies. Reprod Toxicol 5:301–335
62. Zhu P, Huber E, Kiefer F, Gottlicher M 2004 Specific and redundant functions of histone deacetylases in regulation of cell cycle and apoptosis. Cell Cycle 3:1240–1242
63. Tirado OM, Martinez ED, Rodriguez OC, Danielsen M, Selva DM, Reventos J, Munell F, Suarez-Quian CA 2003 Methoxyacetic acid disregulation of androgen receptor and androgen-binding protein expression in adult rat testis. Biol Reprod 68:1437–1446
64. Tirado OM, Selva DM, Toran N, Suarez-Quian CA, Jansen M, McDonnell DP, Reventos J, Munell F 2004 Increased expression of estrogen receptor β in pachytene spermatocytes after short-term methoxyacetic acid administration. J Androl 25:84–94
65. Font de Mora J, Brown M 2000 AIB1 is a conduit for kinase-mediated growth factor signaling to the estrogen receptor. Mol Cell Biol 20:5041–5047
66. Vaczek D 2005 Top 200 prescription drugs of 2004. Pharmacy Times 71:41
67. Reik W, Walter J 2001 Genomic imprinting: parental influence on the genome. Nat Rev Genet 2:21–32
68. Razin A, Kantor B 2005 DNA methylation in epigenetic control of gene expression. Prog Mol Subcell Biol 38: 151–167
69. Anway MD, Cupp AS, Uzumcu M, Skinner MK 2005 Epigenetic transgenerational actions of endocrine disruptors and male fertility. Science 308:1466–1469
70. Heindel JJ 2003 Endocrine disruptors and the obesity epidemic. Toxicol Sci 76:247–249
71. Kanayama T, Kobayashi N, Mamiya S, Nakanishi T, Nishikawa J 2005 Organotin compounds promote adipocyte differentiation as agonists of the peroxisome proliferator-activated receptor γ/retinoid X receptor pathway. Mol Pharmacol 67:766–774
72. Meyer UA, Gut J 2002 Genomics and the prediction of xenobiotic toxicity. Toxicology 181–182:463–466
73. Rushmore TH, Kong AN 2002 Pharmacogenomics, regulation and signaling pathways of phase I and II drug metabolizing enzymes. Curr Drug Metab 3:481–4890
74. Lewis DF, Jacobs MN, Dickins M, Lake BG 2002 Quantitative structure–activity relationships for inducers of cytochromes P450 and nuclear receptor ligands involved in P450 regulation within the CYP1, CYP2, CYP3 and CYP4 families. Toxicology 176:51–57
75. Jacobs MN 2004 In silico tools to aid risk assessment of endocrine disrupting chemicals. Toxicology 205:43–53

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