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Topic 1.4

Biological function and mode of action of the androgen receptor*

Elizabeth M. Wilson

Laboratories for Reproductive Biology, and the Department of Pediatrics and the Department of Biochemistry and Biophysics, University of North Carolina, Chapel Hill, NC 27599, USA

Abstract: Chemical contaminants with antiandrogen or androgen activity have been identified in the environment. The mode of action of these endocrine disruptors derives from their ability to bind to the androgen receptor (AR), a member of the steroid receptor family of nuclear receptors. The AR is a ligand-activated transcription factor with properties unique among members of the steroid receptor family. Binding of endocrine disruptors to the AR impacts its ability to activate or inhibit AR-regulated genes. Most notable of these are the agonist-induced and antagonist-inhibited interdomain interactions that influence AR stability and function. Environmental antiandrogens identified thus far are metabolites of pesticides and herbicides, exerting androgen antagonist effects by blocking AR-induced gene transcription required for male sexual development. Environmental androgens can be precursors of the naturally occurring biologically active androgens testosterone and dihydrotestosterone or result from anabolic steroid use in the livestock industry. They have agonist activity by their ability to bind the AR and mimic the natural hormone, increasing AR-mediated transcription of androgen-responsive genes. The presence of masculinized female fish in polluted rivers indicates the presence of androgen-like pollutants in the environment.

STRUCTURAL AND FUNCTIONAL PROPERTIES OF THE ANDROGEN RECEPTOR

Domain structure of the androgen receptor

The androgen receptor (AR) is encoded by a single gene on the X chromosome at Xq11-12 [1,2]. The AR consists of 919 amino acid residues [3] and occurs as a single form in mammalian cells. The precise amino residue length of the AR can vary, however, due to the presence of polymorphic amino acid repeats. In contrast, most other steroid receptors are coded by more than one gene and are expressed in multiple forms. Previous reports of A and B forms of the AR appear to result from proteolytic breakdown during tissue preparation [4]. AR is sensitive to degradation during its isolation, and in some cases is not protected from degradation by the addition of protease inhibitors [5]. AR degradation is minimized by the binding of high-affinity androgens [6]. The overall structural arrangement of the AR is similar to other steroid receptors with several relatively autonomous domains contributing a specific function. These include the NH₂-terminal region, the DNA-binding domain, and the carboxyl-terminal ligand-binding domain.

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NH₂-terminal region

The AR NH₂-terminal region is comprised of 558 amino acid residues and contains the major transactivation domain [7] referred to as activation function 1 (AF1). This is a poorly defined region that lies within amino acid residues 142–337 [8]. AF1 appears to be the predominant activation region in AR and depends on androgen binding for activation [7].

The NH₂-terminal region contains in addition several amino acid repeat sequences, the most notable being the CAG trinucleotide repeat that codes for a stretch of glutamine residues. The NH₂-terminal region also contains a glycine repeat that has a more constant length of about 23 residues. The function of the glutamine and glycine repeat in AR activity is not known. The CAG repeat region coding for polyglutamine is polymorphic in the normal population. Shorter CAG repeat lengths have an increased association with prostate cancer [9]. The AR CAG repeat length is relatively invariant even in its expanded form [10] and has been used as a marker in human population genetics. CAG repeat expansion to more than 40 glutamine residues results in Kennedy's disease or adult onset spinal bulbar muscular atrophy [11]. While the direct relationship between glutamine repeat length and Kennedy's disease is not understood, the expanded CAG repeat is associated with decreased expression of the AR messenger RNA, resulting in reduced AR protein levels in transfection assays [12]. Trinucleotide repeat expansions in other genes are associated with neurological diseases such as Huntington's disease, the fragile X syndrome, and myotonic dystrophy, where repeat sequences occur in coding or noncoding regions depending on the gene [9]. The AR CAG trinucleotide repeat length has undergone an exponential increase through evolution of the primates back to the lemur [13]. The rat AR sequence has a short glutamine repeat at the position of the human AR CAG repeat, but remarkably, has a CAG repeat expansion at another region of the AR NH₂-terminal domain [14].

The AR has multiple phosphorylation sites that occur predominantly in the NH₂-terminal region and include Ser-Pro sequences [15]. Thus far, specific functions have not been attributed to AR phosphorylation or to phosphorylation of the steroid hormone receptors in general. While all of the steroid receptors contain multiple phosphorylation sites, most studies have failed to define their functional significance.

DNA-binding domain

The central region of the AR is the DNA-binding domain. It consists of amino acid residues 559–624 in human AR. The DNA-binding domain is comprised of two zinc-finger structures typical of steroid receptors [16] and mediates AR binding to DNA as a dimer [17]. The DNA-binding domain binds androgen response element DNA to mediate androgen-induced gene transcription [18,19]. Some of the androgen response elements associated with androgen-regulated genes are simple elements that conform to the consensus sequence 5'GGTACAnnnTGTCT3'. Others have more complex sequences. A number of androgen-regulated genes have been identified that contain simple or complex androgen response elements [20–23], including the androgen responsive regions of prostate specific antigen [24], probasin [25,26], and the *Slp* gene [27].

The DNA-binding domain also functions in interactions with coactivators such as protein inhibitor of activated STAT-1, which increases AR-mediated gene activation [28,29]. Another DNA-binding domain interacting protein is a small nuclear RING finger protein which increased AR transactivation [30].

The DNA-binding domain is contiguous with a hinge region that spans amino acid residues 625–675. The function of the hinge region is not well understood. Spanning the DNA binding and hinge regions is a bipartite nuclear targeting signal composed of two clusters of basic amino acids [31]. In transiently transfected COS cells, androgen binding activates the nuclear targeting signal to direct subcellular localization of the AR from the cytoplasm to the nucleus [7,31]. The subcellular localization of the endogenous AR has been difficult to establish, however. In studies using tissue sections, endoge-

nously expressed AR is nuclear in the presence of androgen, but is not easily detected in the absence of androgen [32], most likely due to its rapid degradation [6]. In normal skin fibroblasts and some cancer-derived cell lines, the AR is nuclear in the absence of dihydrotestosterone (DHT) [33].

Ligand-binding domain

The carboxyl-terminal region from amino acid residues 676–919 makes up the ligand-binding domain. Structural aspects of the ligand-binding domain of many steroid receptors have been elucidated through crystallization studies as recently reported for the AR [34,35]. Crystal structure analysis has revealed multiple alpha helices folded to create a hydrophobic pocket for hormone binding. Binding of the steroid completes the hydrophobic center of the ligand-binding domain and repositions helix 12 [36]. In the presence of agonist binding, a hydrophobic binding surface forms in the ligand-binding domain known as activation function 2 (AF2). AF2 binds the p160 family of coactivators which have histone acetyltransferase activity. Binding occurs through the LXXLL motifs of the p160 coactivators, where L is leucine and X is any amino acid. The AF2 binding surface for some receptors also serves as the binding site for the LXXLL-like sequences present in corepressors. The nature of the ligand bound in the ligand-binding pocket, whether agonist or antagonist, determines the conformation of AF2 to favor either coactivator or corepressor binding.

Steroid-binding specificity and kinetics

The AR binds the biologically active androgens, testosterone, and DHT, with the same apparent equilibrium binding affinity of K_d 0.1–0.3 nM. Yet, DHT is a more effective androgen than testosterone in part because it dissociates more slowly from the AR. The dissociation half-time of DHT is about 3 times slower than that of testosterone [37]. The similar binding affinity and different dissociation rates was supported by differences in androgen association rates [37]. Other steroids such as estradiol and progesterone bind the AR with lower affinity, causing AR nuclear transport, but are less effective in activating reporter vectors in transient transfection studies, requiring high concentrations [6]. Recent studies indicate that the slow dissociation rate of bound androgen results in part from an NH_2 -terminal and carboxyl-terminal (N/C) interaction discussed below.

Androgen antagonists bind the AR and inhibit androgen-induced gene activation. The most notable AR antagonist is hydroxyflutamide, which is the active metabolite of flutamide, a pharmaceutical drug administered for the treatment of prostate cancer. Hydroxyflutamide binds AR with an apparent binding affinity of 175 nM [38], which is 3 orders of magnitude weaker than the binding affinity of testosterone and DHT. Other pharmaceutical antagonists in use in prostate cancer therapy include casodex (bicalutamide) and nilutamide. The mechanism of inhibition of these antagonists is competition for androgen binding and inhibition of AR DNA binding, as shown for hydroxyflutamide [39]. One antagonist, RU56187, binds the AR with an affinity (K_d 0.39 nM) similar to that for testosterone and DHT [38]. However, the dissociation half-time of [3H]RU56187 is 5 min at 35 °C compared to 2.5 h for [3H]R1881 [38]. Thus, one distinguishing feature of AR agonists and antagonists is a more rapid dissociation rate of antagonists. This occurs in part because antagonists fail to induce the N/C interaction [40]. Depending on the binding affinity, antagonists require sufficiently high concentrations to compete for binding of the active androgens to block agonist-induced gene transcription. Environmental antiandrogens bind the AR with relatively low affinity and would be expected to have rapid binding and dissociation kinetics. Environmental antiandrogens, therefore, require relatively high concentrations in accordance with their lower binding affinity to elicit an antagonistic effect.

Androgen receptor stabilization and the N/C interaction

Several years ago, we discovered an NH₂-terminal and carboxyl-terminal (N/C) interaction in the AR [17,40]. The N/C interaction is remarkably specific in that it is induced by ligands that display androgen agonist activity *in vivo*, such as testosterone and DHT [41]. Furthermore, the agonist-induced N/C interaction is inhibited by antagonist binding [40]. Thus, the ligand requirements for the N/C interaction reflect the biological activity of AR as observed *in vivo* suggesting it is important to AR function. Based on studies using mutant receptors that cause the androgen insensitivity syndrome, we proposed a model for an antiparallel dimer of the AR where the N/C interaction directly participates in AR dimerization in association with DNA binding [40,42]. Studies by other laboratories have confirmed the N/C interaction in the AR [43–49] and for other nuclear receptors [50–53].

Recent studies have localized the regions in the AR ligand-binding domain and NH₂-terminal region required for the N/C interaction. Through the use of selective AR mutants that cause the androgen insensitivity syndrome, we showed that the binding site in the ligand-binding domain for the NH₂-terminal region overlaps with the binding site for the p160 coactivators [54]. The coactivator binding site in the ligand-binding domain is known as activation function 2 (AF2). Interaction of the p160 coactivators with AF2 is mediated by multiple LXXLL motifs. We searched for similar motif sequences in the AR NH₂-terminal region. Two motifs were identified, FXXLF and WXXLF, with the sequences ²³FQNLF²⁷ and ⁴³³WHTLF⁴³⁷, where F is phenylalanine and W is tryptophan. Each binding motif is predicted to form an amphipathic alpha helix and interact with AF2 in the presence of androgen to mediate the N/C interaction [55]. An interesting aspect of these studies is that the FXXLF motif is present in several previously reported AR coactivators [56]. The studies indicated that the FXXLF interacting motif is specific for the AR.

Because of the overlapping binding sites present in AF2 for the LXXLL motif of p160 coactivators and the FXXLF motif in the AR, we determined whether competitive inhibition occurs. We showed that the N/C interaction inhibits the recruitment of p160 coactivators [57]. Thus, one function of the agonist-induced N/C interaction may be to limit AR activation through AF2. On the other hand, there may be a temporal sequence of interactions between AF2 and the FXXLF and LXXLL motifs that does not preclude AR activation by p160 coactivators through the AF2 domain. Other coactivators and interacting proteins that contain the FXXLF motif may interact with the AF2 binding surface at different times during the process of AR-mediated gene activation.

Recent studies showed that the androgen-dependent N/C interaction mediated by the FXXLF and WXXLF motifs is required for the activation of several naturally occurring androgen-regulated genes including PSA and probasin [58]. This was in striking contrast to the mouse mammary tumor virus long terminal repeat and *Slp* androgen-responsive regions, which showed less dependence on the N/C interaction for activation. It is not known what distinguishing properties of these androgen-responsive regions require an AR N/C interaction for transactivation.

With few exceptions, compounds with AR agonist activity *in vivo* induce the N/C interaction. This includes the lower-affinity anabolic steroids such as oxandrolone and fluoxymesterone, which bind AR with an equilibrium binding affinity of 62 and 44 nM, respectively [38]. These are potent androgens *in vivo*, supporting an *in vivo* role for the N/C interaction in AR activity. This contrasts other steroids such as estradiol and progesterone, which bind AR with a similar moderate affinity, but which are ineffective in activating the AR *in vivo*. Specificity of the N/C interaction for agonists and antagonists makes it a useful assay to screen compounds for endocrine disruptor activity. The two hybrid mammalian cell assay used for the N/C interaction was recently described in detail [59]. It utilizes the GAL4 DNA-binding domain fusion protein with the AR ligand-binding domain and the VP16 transactivation domain fusion protein with the AR NH₂-terminal and DNA-binding domains. The reporter vector contains multiple binding sites for the GAL4 DNA-binding domain and the luciferase gene for detection.

Nuclear coactivators of the androgen receptor

Many proteins have been reported to interact with the AR and function as AR coactivators. Some of these include Ets [60], c-Jun [61,62], TFIIF [63], TFIIF [64], retinoblastoma protein [65], CREB-binding protein [66], BAG-1L [67], ARA70/ELE1 α [68–70], ARIP3 [71], p160s [72,73], ARA160 [74], ARA54 [75], ARA55/Hic5 [76,77], β -catenin [78,79], FHL2 [80], HBO1 [81], cyclin E [82], cyclin D1 [83], PDEF [84], BRCA1 [85], P-TEFb [86], caveolin-1 [87], and ARA24 [88]. Many of the reports utilized yeast two hybrid screening methods to detect interacting proteins and made use of transient transfection studies to demonstrate increased transcriptional activity mediated by the AR. There are as yet few or no studies that directly link an AR interacting protein with AR functional activity *in vivo*. Knock-out studies of the p160 coactivators in mice have failed to significantly impact male reproductive development and function [89–91]. This type of study is complicated, however, by the compensating effects of other coactivators that may become expressed at increased levels. Another complication in determining the functional significance of an AR interacting protein results from limitations of conditions used for transient transfection assays. We [56] and others [92] have observed that the use of balancing DNA additions in transient cotransfection assays inhibit AR transcriptional activity in controls, but not necessarily in samples expressing the coactivator being tested. The mechanism of inhibition has not been defined, but may include inhibition of AR expression [92] or inhibition of AR functional activity. The result is an apparent increase in AR-mediated transactivation by the putative coactivator under study, which may not be observed without the inhibitory effect. Conclusions regarding many of the interacting proteins that show apparent increases in AR transcriptional activity, therefore, require further study to clearly establish their role in AR function.

ANDROGEN RECEPTOR GENE MUTATIONS

Androgen insensitivity syndrome

Male sexual development depends on androgen-induced gene transcription. This occurs in the human embryo between 8 and 12 weeks of gestation. At this critical period, the external genitalia of the male forms, and interference with androgen action at this time results in different degrees of incomplete masculinization. At birth, the androgen-insensitive genetic male external genital phenotype can vary from near complete masculinization to ambiguous genitalia, or an apparent normal female. The requirement for the AR in this process has been well documented in the androgen insensitivity syndrome. In this syndrome, 46XY genetic males have a mutation in the AR gene, which results in different degrees of loss of AR function depending on the specific mutation [93]. There have been approximately 230 different mutations identified in the AR gene that cause androgen insensitivity. These are summarized at <www.mcgill.ca/androgendb/>.

AR mutations that cause androgen insensitivity include partial or complete AR gene deletions [94,95] and nonsense or missense mutations [96–99]. Frameshift mutations that result in the premature stop codon sequence destabilize the messenger RNA and can result in a truncated AR protein [100]. In cases of partial androgen insensitivity, the function of the AR is not entirely disrupted, resulting in different degrees of incomplete virilization in the newborn [101]. Thus, the full function of the AR is required for male sexual development to occur. Exposure to sufficient levels of environmental antiandrogens could have a similar effect of inhibiting AR-mediated gene transcription with partial or complete interference of male sexual development *in utero*. The androgen insensitivity syndrome, therefore, unequivocally demonstrates the requirement for the AR in male sexual development. Similar phenotypic disruptions could result by interrupting AR-mediated gene activation by the presence of environmental androgen antagonists.

Androgen receptor in prostate cancer

Androgens are required for prostate growth and likely have role in excessive tissue growth associated with benign prostate hyperplasia and prostate cancer [33]. Accordingly, antiandrogens are frequently used in the treatment of prostate cancer. Antiandrogens at moderate concentrations block androgen-induced stimulation of the prostate [102]. However, the lower-affinity environmental antiandrogens or androgens likely have little impact on benign prostatic hyperplasia or prostate cancer. Some reports have suggested that inappropriate exposure in utero to environmental estrogenic toxicants can increase prostate growth and development later in life [103], although the significance of lose-dose effects to environmental contaminants has recently been challenged [104]. Adult males are exposed to relatively high circulating androgen levels that would not be influenced by environmental antiandrogens or androgens. It should nevertheless be considered that some stages of prostate cancer are sensitive to low concentrations of androgen through mechanisms not yet understood [33,105]. In patients treated with androgen withdrawal therapy, exposure to androgens could be detrimental. High p160 coactivator expression has been reported in recurrent prostate cancer in the androgen-withdrawn patient, which may contribute to an increased sensitivity to androgen [105]. It is unlikely that environmental androgens or antiandrogens would be in sufficiently high concentrations to impact these examples of increased prostate growth.

RISK ASSESSMENT OF ANTIANDROGENS AND ANDROGENS AS ENVIRONMENTAL ENDOCRINE DISRUPTORS

Environmental antiandrogens

A number of chemicals used in agricultural weed and pest control have been identified as precursors to environmental antiandrogens. The pesticides *p,p'*-DDT [1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane] [106] and methoxychlor [2,2-bis (*p*-methoxyphenol)-1,1,1-trichloroethane] [107] are metabolized to more potent compounds with androgen antagonist activity. The AR antagonist activity of *p,p'*-DDT derives from its metabolite *p,p'*-DDE [1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethylene], which binds the AR with an apparent equilibrium binding affinity of K_d 3.5 μ M [106]. The potency of *p,p'*-DDE as an endocrine disruptor is increased because of its long biological half-life, which was estimated to be only slightly less than the average human life span. The long half-life of *p,p'*-DDE derives from its solubility in fat stores from where it is released during mobilization of fat and lactation. The active metabolite of methoxychlor is HPTE [2,2-bis (*p*-hydroxyphenol)-1,1,1-trichloroethane], which binds AR with an equilibrium binding affinity of K_d 1.4 μ M [107]. HPTE has a high turnover rate in the body and thus a lower potential as an endocrine disruptor in human development and health. While *p,p'*-DDT is no longer in use in the United States as a pesticide, methoxychlor is an actively used pesticide and thus could be a source of antiandrogen activity in the environment. The fungicide vinclozolin is currently in use in the United States. Vinclozolin [3-(3,5-dichlorophenyl)-5-methyl-5-vinylloxazolidine-2,4-dione] is metabolized to M2 (3',5'-dichloro-2-hydroxy-2-methylbut-3-enamide), an androgen antagonist that binds the AR with an affinity of K_d 9.7 μ M [39,108]. Administration of vinclozolin to pregnant rats inhibits male reproductive tract development in male offspring, indicating its androgen antagonist activity [109,110].

Other potential environmental antiandrogen precursors include procymidone [*N*-(3,5-dichlorophenyl)-1,2-dimethylcyclopropane-1,2-dicarboximide], a fungicide with antiandrogen activity in experimental animals [111]. The active metabolite of procymidone remains uncharacterized. Other environmental contaminants disrupt male reproductive development through mechanisms that are thought to be independent of the AR. These include di(*n*-butyl) phthalate, a widely used commercial plasticizer present in fatty foods [112]. Dibutylphthalate is metabolized to the active mono (*n*-butyl) phthalate and inhibits testosterone biosynthesis [112,113].

The reported increased incidence of male reproductive tract abnormalities in the human population in some parts of the world has raised concerns about the role for environmental contaminants with

antiandrogen activity. The question of declining sperm counts worldwide or regionally was recently addressed. Most evidence supports regional declines, but controversy remains over the reliability of historical data [114]. The role of environmental antiandrogens in inhibiting spermatogenesis has not been addressed, but could reflect global effects of environmental contaminants. Another male developmental abnormality of incomplete masculinization with reported increased frequency is hypospadias deformity, where the urethra opens on the shaft or base of the penis rather than at the tip [115]. Hypospadias is a relatively common developmental abnormality of the human male fetus, occurring at a rate as high as 40 in 10 000 newborn male infants [115,116]. In the United States, the incidence of hypospadias reached a high level in the 1980s, but has since declined [117]. It is not clear what role endocrine disruptors have in the etiology of hypospadias in the newborn.

Environmental androgens

Effluent from paper mills flowing into a Florida river was postulated to contain substances with androgen activity based on the masculinization of entire populations of *Gambusia affinis holbrooki* female mosquitofish [118]. One active substance was identified as androstenedione present in the river at concentrations of 0.14 nM [119]. Previous studies showed that short exposure to higher concentrations of androstenedione masculinized the anal fin or gonopodium used in male reproduction [118]. Thus, aquatic wildlife in areas of paper mill discharge are at increased risk of reproductive tract abnormalities due to the presence of androgen precursors in the river water [120]. It was postulated that the abundant phytosterols from pine tree oils, including β -sitosterol, campesterol and stigmastanol, act as substrates for bacteria like the common bacterium *Mycobacterium sp.* [119], which was shown to metabolize phytosterols to androstenedione [121,122]. Other studies confirmed the presence of androgen activity in river water downstream of a paper mill, but as yet no additional specific compounds have been identified [123]. Ongoing studies of river water extracts indicate the presence of additional compounds with androgen activity. Preliminary evidence indicates that their structure differs from the known steroids. There is also recent evidence that other substances with androgen activity are present in the control river [124].

Another potential source for environmental androgens is excreted anabolic steroids administered to cattle to improve beef production [125]. The anabolic steroid trenbolone acetate is rapidly converted in vivo to 17 β -trenbolone, an active metabolite with androgen activity. In a number of in vitro assays, trenbolone has a potency similar to the most active androgens known such as DHT and the synthetic androgen R1881 (methyltrienolone) [125]. Detection of reproductive abnormalities in fish exposed to effluent from feed lots has raised questions whether the masculinizing effects result from excretion products that contaminate the water. This type of environmental contamination by pharmaceutical byproducts in water sources may be widespread resulting from human or livestock drug use. Such contamination allows for possible exposure to high-affinity compounds, which if not eliminated by water purification procedures, can expose wildlife and human populations.

An environmental threat, therefore, exists, especially to aquatic wildlife that live in contaminated waters that contain effluent from paper mills or feedlots, as evidenced by the increased incidence of reproductive abnormalities. It is yet unclear to what extent these androgen precursors, anabolic steroids, or metabolites impact the human population. The presence of high-affinity compounds or androgen precursors lowers the exposure requirements for a detrimental effect. Further study of these contaminants with high affinity for the AR will reveal the overall threat to wildlife and human populations.

Risk assessment

Several criteria must be considered in the risk assessment of environmental antiandrogens and androgens. These include (a) exposure levels, (b) in vivo metabolic activation, (c) biological half-life of the

active metabolites, (d) binding affinity and activity for the AR, and (e) the developmental stage of exposure.

- (a) The level of exposure of the human population to environmental androgens or antiandrogens in most cases is not known. The relatively low binding affinities of the environmental antiandrogens discovered thus far would require significant exposure levels for an antagonistic effect to occur. Exposure to endocrine disruptors with antiandrogen activity may be an occupational hazard and thus limited to select populations involved in chemical production or farming practices. In contrast, the presence of high-affinity compounds derived from pharmaceutical and personal care products reduces the exposure requirements for a detrimental impact.
- (b) Metabolic activation was observed with all of the environmental antiandrogens derived from pesticides and herbicides. This indicates the importance of screening with assay techniques that allow for metabolic processes to occur. *In vitro* binding assays using inactive parent compounds and isolated receptor preparations could preclude the identification of androgen antagonists or agonists since metabolic conversion may not occur. Metabolic activation necessitates the use of biological systems such as mammalian cell lines that utilize the correct metabolic enzyme pathways. For example, in yeast-based assays, metabolic conversion may not occur or may occur through alternative pathways not applicable to mammalian cells. Ideally, *in vivo* whole animal experiments may be required to demonstrate the activity of a compound. However, such an initial screening approach would be expensive and laborious. In the case of vinclozolin, mammalian cell-based assays displayed the same metabolic conversions that occur in the whole animal [39]. For a clear documentation of the inhibitory effects, the active metabolites must be identified and confirmed.
- (c) Long half-lives of active endocrine disruptors can result from high fat solubility, a property applicable to most steroidal compounds that interact with the ligand-binding domain of steroid receptors. When fat stores are mobilized, the potential impact to human health increases. In addition, steroids or steroid-like compounds can accumulate in wildlife species to be passed up the food chain or to increase the body load to levels sufficient for inhibitory or stimulatory effects to occur.
- (d) The environmental antiandrogens discovered thus far have relatively low binding affinities for the AR. This necessitates relatively high exposure levels for an inhibitory effect, which may only occur during occupational exposure. On the other hand, the presence of pharmaceutical and personal care products such as cholesterol-lowering drugs and steroids like estrone in water sources, raises new threats of the presence of high-affinity steroids and ligands that would require lower exposure levels for a detrimental effect. It was shown that some pharmaceutical products detected in the environment are associated with populations of feminized male fish, thus broadening the involvement of environmental contaminants in male reproductive abnormalities [126]. The detection of pharmaceutical and personal care products in the environment, which characteristically have high binding affinities for the AR, reduces the exposure levels required for a detrimental effect.
- (e) Risk assessment should consider the developmental period of exposure. The developing male fetus is sensitive to the presence of antiandrogens since the male embryo depends on androgen-induced gene transcription during the critical period of male sexual development [127]. Periods of increased fetal sensitivity to exposure to antiandrogen activity from the fungicide vinclozolin were identified in a study of rats. The most sensitive period to the antiandrogenic effects of vinclozolin was rat gestational day 16 and 17, with the sensitive period ranging from gestational day 14 to 19, the time of male sexual development [128]. The relatively weak binding affinities of the known environmental antiandrogens for the AR necessitates relatively high exposure levels for a biological impact. It remains to be established whether any of the environmental antiandrogens identified thus far reach levels that are sufficient to interfere with normal human reproductive de-

velopment. It was recently suggested that the lack of feedback regulation at the hypothalamus and pituitary by fetal androgens during development may increase the inhibitory effects of an antiandrogen [129]. In this case, androgen production by the fetal testis is induced by placental human chorionic gonadotropin. This could prevent a compensatory increase in androgen production in the presence of an antiandrogen, which could result in a more pronounced inhibitory effect.

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