Sex steroid effects at target tissues: mechanisms of action

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Wierman ME. Sex steroid effects at target tissues: mechanisms of action. Adv Physiol Educ 31: 26–33, 2007; doi:10.1152/advan.00086.2006.—Our understanding of the mechanisms of sex hormone action has changed dramatically over the last 10 years. Estrogens, progestins, and androgens are the steroid hormones that modulate reproductive function. Recent data have shown that many other tissues are targets of sex hormones in addition to classical reproductive organs. This review outlines new advances in our understanding of the spectrum of steroid hormone ligands, newly recognized target tissues, structure-function relationships of steroid receptors, and, finally, their genomic and nongenomic actions. Sex-based specific effects are often related to the different steroid hormone milieu in men compared with women. Understanding the mechanisms of sex steroid action gives insight into the differences in normal physiology and disease states.

Steroid receptors; genomic and nongenomic actions

Sex Steroid Hormones, including estrogens, progestins, and androgens, traditionally have been defined by their role in normal reproductive function. Estradiol and progesterone were considered the major sex hormones produced by the ovary and testosterone produced by the testis. Steroid hormones, however, are also produced in locally by peripheral conversion in target tissues such as fat and the liver. These hormones may act in a paracrine manner or circulate to act at target tissues in an endocrine fashion. Recently, researchers have challenged the classic dogma about how sex hormones work. Information concerning their variability in ligand availability, newly recognized alternative forms of sex steroid receptors, previously unrecognized targets of steroid hormones, and different modes of genomic and nongenomic actions have altered our knowledge of normal physiology. These data have, in turn, given new insights into pathological states. An understanding of this new information can shed light into sex-based differences in disease and responses to therapeutic interventions.

Sex Steroid Hormones: Their Role as Reproductive Hormones

Classically, sex steroid hormones have been defined by their role in normal reproductive function. In the hypothalamic-pituitary-gonadal axis, gonadotropin-releasing hormone is secreted in an episodic fashion from the hypothalamus to activate the production of gonadotropins, luteinizing hormone (LH), and follicle-stimulating hormone (FSH) from the anterior pituitary (36, 37) (see Fig. 1). LH and FSH are released in a pulsatile manner to act at the gonad to control both gametogenesis (spermatogenesis or oogenesis) as well as steroidogenesis. The major sex hormones estradiol, progesterone, and testosterone are secreted in response to gonadotropins and, in turn, feedback at the level of the hypothalamus and pituitary to control normal reproductive function.

What Are the Relevant Sex Hormone Ligands?

Historically, we were taught that androgens are “male” hormones and estrogens are “female” hormones. Most of the physiological research concerning the roles of androgens in male reproductive function, however, was performed using the aromatizable androgen testosterone(10). Testosterone and the weaker adrenal prohormones DHEA and dehydroepiandrosterone sulfate can be converted by aromatization into estrogens (Figs. 2 and 3). Common estrogens include estradiol, estrone, and estriol. Progesterone is the natural progestin. Recent studies (7, 19, 34, 40, 44) of naturally occurring mutations in the estrogen receptor (ER) or aromatase deficiency in humans as well as genetic mouse models have reoriented the field as to the critical importance of estrogen action in males as well as in females.

The major estrogens include estradiol, estrone, and estriol. Estradiol is the major estrogen produced by the ovaries, but estrogens are also produced locally in targets such as adipose tissue. In postmenopausal women, the effects of locally produced androgens and estrogens may explain some of the excessive risks of combined conjugated estrogen with daily progesterin hormonal therapy when given broadly to overweight or obese women (40). The major progestin is progesterone, which is made predominantly by the ovaries but also made locally in tissues. Progestins may have important local effects to modulate both sexual behavior and neurotransmitter function in the central nervous system (25, 41).
Model Systems to Study Steroid Hormone Actions

The complexities of sex hormone action have been elucidated by a variety of research approaches and use of different model systems. Initial biochemical reconstitution studies defined the structure-function relationships of ligands and their receptors and other important proteins involved in transcriptional regulation. These type of studies, however, were often performed in nonphysiologically relevant cell systems in an “add-back” approach and may not reflect the target tissue environment. Additional work has been performed in more physiological relevant tissue specific cell systems but often with immortalized or tumorous tissue. Fewer data have been derived from primary cultures of cells that are targets of sex steroid hormones, but when these data are available, they can be used to confirm or refute data in other systems. Parallel studies in animal models, primarily in the rodent and primate, have given insights into the mechanisms of sex steroid action. Both transgenic mouse overexpression models as well as knockout models of steroid hormone ligands or receptors have identified previously unsuspected targets of sex hormone action and changed our understanding of normal physiology. Finally, studies in humans, both across normal development and with diseased states, have provided important data concerning the potential clinical use of sex hormone agonists and antagonists.

Sex Hormone Targets

Until recently, researchers assumed that the targets for sex hormones were primarily the reproductive organs: the breast, female reproductive tract (uterus and ovary), and male reproductive tract (testes and epididymis) (37, 47). Bone was known to be a target of sex hormones based on the data that gonadectomy of either sex resulted in osteoporosis and sex-specific hormonal replacement restored bone structure and function (23). An expanded list of sex hormone targets became apparent when investigators examined the phenotypes of naturally occurring mutations in humans and genetically altered mouse models. Deficiency of aromatase (the enzyme that converts testosterone to estradiol) or knockout of the ER, progesterone receptor (PR), or androgen receptor (AR) in mice showed tissue-specific deficits (6 – 8, 10, 15, 26, 34). Together, this research suggested that sex steroid hormones function in an expanded list of target tissues (Fig. 4). These include the vascular system, central nervous system, gastrointestinal tract, immune system, skin, kidney, and lung. An understanding of the tissue-specific roles of gonadal hormones is important when predicting the benefits or risks of replacing natural ligands or use of steroid hormone antagonists in humans.

Sex Hormone Actions in the Vasculature

Recent investigation has shown the importance of sex hormone action in the vasculature in both sexes (28 –30). Research has documented the presence of ERs, PRs, and ARs in vascular endothelial cells, smooth muscle cells, and cardiomyocytes as targets of sex hormone ligands. Estrogen administration has...
been shown to improve vascular reactivity, increase nitric oxide production, decrease free radical production, and prevent programmed cell death in normal vasculature. In contrast, in the diseased vessel, a different gene program may be activated in response to estrogen administration that promotes plaque destabilization and thrombosis through the activation of metalloproteinasises. Importantly, studies have confirmed that there is a dose-response relationship to various sex steroid ligands in different tissues. These basic studies may give insight into the unexpected toxicities when combined conjugated equine estrogens and daily progestins were given broadly to postmenopausal women in Women’s Health Initiative trials (1, 32, 35, 45).

**Sex Steroid Receptors**

Steroid receptors have been cloned and characterized (12). There are two ERs (ERα and ERβ), two PRs (PRA and PRB), and one AR (see Fig. 5). Sex steroid receptors represent one category of nuclear receptors, with the two other categories including class II receptors (e.g., vitamin D, thyroid hormone, peroxisome proliferator, and retinoid receptors) and orphan receptors (e.g., steroidogenic factor-1 and estrogen-related receptor). Classically, it was thought that two molecules of each steroid receptor bound by the ligand then interacted with target DNA through palindromic hormone response elements (HREs) to act as transcription factors to control gene expression. In contrast, class II receptors act as heterodimers with retinoid X receptors on direct repeat HREs, whereas orphan receptors act independent of the ligand as monomers on half-site HREs.

The evolution of this large family of nuclear receptors has been complex with 250 receptors in Drosophila, 21 receptors in C. elegans, and 48 receptors in the human (2, 11). Similarly, the evolution of ligands has diverged across evolution with no ligands and 250 orphan receptors in Drosophila, 23 ligands and 250 orphan receptors in C. elegans, 1 ligand and 20 orphans in human (5, 11, 12). The midregion of the molecule contains the DNA binding region (DBD), followed by a hinge region and then the ligand binding domain (LBD). Dimerization interfaces are located in the mid- and COOH-terminal regions. The COOH terminus of the molecule within the LBD contains the AF-2 domain, which is another transcriptional activation region that is dependent on the ligand. Biochemical approaches have dissected the structure/function of the steroid receptors, which was confirmed when the crystal structure of the various domains with and without ligands was solved. Each steroid receptor is actively modulated by interactions with various steroid agonists compared with antagonists and with many other proteins to allow correct transcriptional activation or repression.

**New Insights Into Transcriptional Control by Steroid Hormones**

It was initially shown that promoters of genes regulated by sex hormones contained palindromic HREs in the 5′ flanking region that acted as binding sites for liganded steroid receptors (5, 11, 12). The ligand (e.g., estradiol) was shown to circulate in the bloodstream, diffuse into cells, and interact with its cognate receptor in the cytoplasm or nucleus to alter the conformational state (Fig. 7, direct). The liganded steroid receptor was then shown to recognize these HREs on promoters of target DNA to directly bind this DNA as a transcription factor and ultimately increase gene expression. However, as more physiologically relevant gene promoters of sex steroids were identified, it became apparent that many, if not most, promoters lacked consensus HREs in their 5′ flanking regions. Some HREs are in 3′ untranslated regions or far distant to the coding region. In addition, it has become apparent that liganded steroid receptors may function in an alternative mechanism that involves protein-protein interactions to either augment or block

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**Fig. 5. Family of nuclear receptors (NRs).**

<table>
<thead>
<tr>
<th>GR</th>
<th>Glucocorticoid</th>
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<tbody>
<tr>
<td>PR</td>
<td>Progesterone</td>
</tr>
<tr>
<td>AR</td>
<td>Androgen</td>
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<tr>
<td>ER</td>
<td>Estrogen</td>
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**Class II Receptors**

| VDR, PPAR |
| TR, FXR |
| RXR, LXR |
| RAR, PXR |

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**Orphan Receptors**

| NGFI-B |
| SF-I |
| ERR |
| RevERB |

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**Palindrome HREs**

| AGAACA-N3-TGTTCT |
| AGGTCA-N3-TGACCT |

**Direct Repeat HREs**

| ACGGTCA-N1-5-AGGTCA |

**Halfsite HREs**

| AAA-ACGGTCA |
| TCA-AGGTCA |
the effects of other transcription factors bound to promoter DNA (see Fig. 7, indirect). This alternative process of sex steroid action has been shown to be important in the endometrium, where the ER modulates the activities of members of the activating protein-1 family, including Fos and Jun (8, 18). Similarly, in bone cells, the liganded ER modulates gene function by cross talk with NF-κB proteins on relevant target genes (20, 24, 33). This added complexity of the genomic actions of sex hormones must be considered when questioning which are the relevant hormonal targets when sex hormones are abolished or replaced in normal physiological and pathophysiological states.

**Agonists and Antagonists**

Prior work has suggested that naturally occurring or synthesized sex hormone ligands acted as pure agonists or antagonists. Research into the mechanisms of structure-function relationships of the ligand-bound steroid receptor has altered this dogma and now can explain how compounds can be mixed agonist/antagonist or partial (e.g., tamoxifen and raloxifene) or pure (e.g., ICI-182.780). The crystal structure of agonist- versus antagonist-bound ERs demonstrated the distinct differences in how specific ligands fit into the LBD pocket of the receptor and therefore recruit different types of coadaptor proteins to increase or decrease the efficiency of gene transcription (39).

**Coregulators Contribute to Genetic Mechanisms of Sex Steroid Actions**

In the 1990s, many groups contributed to the insight that steroid receptors mediate their transcriptional activities by recruiting a cohort of docking and adapter proteins. The first major group to be characterized were the members of the p160 coactivator family (5, 14, 43). The steroid receptor complex (SRC) family (SRC-1, -2, and -3), cAMP response enhancer protein binding protein (CBP)/p300, and p300/CBP-associated factor were identified as acetyl transferases. In the presence of agonist-bound steroid receptors, these coactivators are recruited to the DNA to allow histone acetylation and unwinding of the DNA to promote more efficient gene expression. Since that time, a long list of coadaptor proteins have been identified that have enzymatic activities including ligases, ATPases, and methylases as well as proteins that serve as cell cycle regulators, RNA helicases, and docking proteins to bridge to basal transcription factors (see Table 1).

**Table 1. Diverse functions of coregulators**

<table>
<thead>
<tr>
<th>Function</th>
<th>Coregulator</th>
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<tbody>
<tr>
<td>Acetyltransferases</td>
<td>SRC/p160, CBP/p300, and pCAF</td>
</tr>
<tr>
<td>Ubiquitin ligases</td>
<td>E6-AP</td>
</tr>
<tr>
<td>ATPases</td>
<td>BRG-1</td>
</tr>
<tr>
<td>Methylases</td>
<td>CARM-1 and PRMT-1</td>
</tr>
<tr>
<td>RNA transcription</td>
<td>SRA</td>
</tr>
<tr>
<td>Cell cycle regulators</td>
<td>Cdc-25B</td>
</tr>
<tr>
<td>RNA helicases</td>
<td>P72</td>
</tr>
<tr>
<td>Direct contact with basal</td>
<td>TRAP/DRIP/mediator</td>
</tr>
<tr>
<td>transcription factors</td>
<td></td>
</tr>
</tbody>
</table>

Fig. 6. Modular structure of steroid hormone receptors. N-domain, NH2-terminus domain; C, COOH-terminus domain.

Fig. 7. Transcriptional action by liganded SRs. TF, transcription factor.

**Refresher Course**

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When the p160 family of coactivator proteins was investigated, it was discovered that they are also modular proteins with domains that serve specific functions (11, 14, 43) (see Fig. 8). The NH2 terminus contains a basic helix-loop-helix and Per-Arnt-Sim domain that can interact with a coactivator or an ATP-chromatin remodeling complex. The steroid interaction (SR) box domain is the region of the molecule that binds to nuclear receptors. It contains an \( \alpha \)-helix domain with the LXXLL motif, which interacts with the hydrophobic pocket in AF-2 (46). The COOH terminus contains two activation domains (AD1 and AD2). AD1 interacts with p300/CBP (with histone acetylase activity), and AD2 interacts with coactivator-associated arginine methyltransferase 1 (a coadaptor with histone methyltransferase activity).

In addition to coactivators, corepressors have been characterized. Small modulator of repression of thyroid hormone (SMRT) and nuclear component of repression (NCOR) are the prototypes for this class of proteins (14, 16, 17, 42). Antagonist-bound sex steroid receptors or agonist-bound thyroid hormone receptors recruit these corepressors rather than coactivators. These corepressors then activate a family of histone deacytelases (HDACs), which result in chromosomal deacytelation and failure to recruit the basal transcription machinery and inhibition of gene expression. This is in contrast to the effects with coactivator recruitment in the presence of agonists, which results in efficient transcriptional activation (Fig. 9).

The prototype corepressors, SMRT and NCOR, are also modular proteins (11, 17) (see Fig. 10). Each contains multiple repression domains (RD1, -2, and -3) that repress transcription by recruitment of HDACs either directly or indirectly. Steroid receptor interaction domains (RID1 and RID2) are the sites of binding to steroid receptors through a recognition sequence called the CoRNR box (IXXI/VI with an extended sequence). A deacytelase activation domain region in the corepressors is important for the activation of HDAC3.

This process of sex steroid-mediated transcriptional activation or repression is quite complex at the molecular level. In addition to many proteins directly or indirectly binding liganded steroid receptors, each protein may be posttranslationally altered to promote or prevent histone modification and chromosomal remodeling (14, 21, 43). Does this newly identified cohort of coadaptor proteins play a specific role in physiology or disease? These studies are ongoing, but there are a few examples of cell-specific patterns of coadaptor gene expression. For example, SRC-2 appears to have specific roles in the endome
trium apart from the other SRC family members (31, 42). Investigators are examining changes in the expression of co-adaptor proteins in disease progression. For example, tamoxifen is an antagonist in breast cancer cells in the presence of corepressors (13). Studies have shown that tamoxifen-resistant MCF-7 breast cancer cells have decreased levels of the corepressor NCOR. Theoretically, lack of corepressor protein expression may allow coactivators to be recruited to antagonist-bound steroid receptors and convert the antagonist to an agonist effect. Samples from breast cancers are under analysis to determine if alterations in coadaptor expression may underly the loss of clinical responses to selective ER modulator drugs.

**Nongenomic Actions of Sex Steroids**

In addition to the classic genomic effects of sex steroids, recent data have shown the importance of acute nongenomic effects (4, 9, 11, 22, 29, 38, 45) (Fig. 11 and Table 2). Sex steroids may act in a variety of ways to modulate cellular activity (4). Studies have provided evidence of actions of estrogens and progestins to directly alter plasma membrane fluidity; however, at micromolar concentrations. Research in the central nervous system and vascular systems has shown the ability of steroids to interact at the plasma membrane with non-nuclear receptors such as ion channels and G protein-coupled receptors (GPCRs). In these systems, the effects are insensitive to antagonists. An explosion of research is dissecting the relevant effects of membrane-bound steroid receptors, which only represent ~2% of the steroid receptor pool but can impact on physiological processes. Potentially relevant membrane-bound steroid receptors have been shown for ERα, ERβ, PR, and AR. In addition, ligand-bound steroid receptors can be modified whether in the plasma, cytoplasmic, or nuclear compartments by alterations in intracellular signaling cascades, which alter serine or threonine phosphorylation on these receptors and indirectly alter cellular function (3, 9, 21). These effects are mediated by the NH2 terminus of the steroid receptor molecule and often involve AF-1 (4, 11) (see Fig. 6).

**Nongenomic Effects of Estrogen Via GPCRs**

In vascular cells, estrogen may interact with plasma-bound GPCRs to induce acute effects on intracellular signaling.

**Table 2. Nongenomic actions of sex steroids**

<table>
<thead>
<tr>
<th>Nonreceptor-mediated actions at the plasma membrane</th>
<th>Membrane fluidity: ligand at millimolar concentrations</th>
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<tbody>
<tr>
<td>Steroid activation of non-nuclear receptors at the plasma membrane</td>
<td>Ion channels and G protein-coupled receptors: insensitivity to antagonists</td>
</tr>
<tr>
<td>Rapid signaling through membrane-bound steroid receptors</td>
<td>2% of pool, estrogen receptor-α or -β, or progesterone receptor</td>
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</tbody>
</table>
through cAMP and can be blocked by pertussis toxin (4, 22) (Fig. 12). This signaling can modify Src, which phosphorylates the EGF receptor (EGFR) and releases metalloproteinases, which trigger the release of heparin-bound EGF ligand to active EGF to augment the tyrosine receptor kinase EGFR. These steps can be blocked with protein phosphatase 2 or CRM-197 (see Fig. 12). Ligand-stimulated EGFR interacts with the docking proteins of Sos and Shc to eventuate in the activation of the Ras/Raf/MEK/ERK signaling system. This complex modulation of multiple intracellular signaling systems can then have both acute, nongenomic effects or chronically modify gene expression. Each cell or target of sex steroid action may have a different complement of membrane receptors and responses to ligands to mediate cell-specific effects in normal physiology and altered complements in disease states.

Summary

Thus, our current understanding of tissue-specific effects of sex steroids has evolved over the past decade. In addressing a potential sex-based or target-specific response, one must account first for ligand availability, i.e., estrogen, progestin, or androgen. Then, one must ask what are the steroid receptor expression profiles for that tissue. In each cell, the promoter-specific response is defined by the promoter organization. New research is characterizing the complement of coregulators in normal cells and disease states. The final word is out on whether there are cell-specific coregulators. In addition, it is hypothesized that the complement of proteins or their activation state is altered in the transition from normal to disease states. Finally, the information concerning the role of sex steroids to act in a nongenomic fashion, cross talking with membrane receptors and multiple intracellular signaling pathways, suggests that our true understanding of sex steroid effects is still not complete. We await with excitement the further unraveling of the complexities of sex steroid action in normal physiology and disease states and the insight this research will provide to new therapeutic options in the future.

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