

# Role of androgens in female genital sexual arousal: receptor expression, structure, and function

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**Objective:** In women, androgens modulate the physiological function of many reproductive and sexual organs, including the ovaries, uterus, vagina, oviducts, clitoris, and mammary gland. In this article, we review the mechanisms of androgen action and discuss new data on the effects of androgens in vaginal and clitoral tissues.

**Main Outcome Measure(s):** In this study, we characterized the androgen receptor expression in rabbit vaginal tissues from control and ovariectomized animals treated with or without androgen replacement therapy. We investigated the effects of androgen deprivation and replacement on the expression and activity of nitric oxide synthase and arginase and on vaginal smooth muscle contractility.

**Result(s):** Androgens enhanced nitric oxide synthase activity and down-regulated arginase activity in proximal vagina. Estrogens down-regulated nitric oxide synthase activity and increased arginase activity in distal vagina. Androgens facilitated vaginal smooth muscle relaxation to electric field stimulation and vasoactive intestinal polypeptide, whereas estrogens attenuated vaginal tissue relaxation to electric field stimulation and to vasoactive intestinal polypeptide.

**Conclusion(s):** These observations suggest that androgens may play an important role in modulating the physiology of vaginal tissue and contribute to female genital sexual arousal. (*Fertil Steril*® 2002;77(Suppl 4): S11–8. ©2002 by American Society for Reproductive Medicine.)

**Key Words:** Androgen receptor, genital sexual arousal, nitric oxide synthase, sexual dysfunction

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Genital sexual arousal in women is characterized by increased genital blood flow, which leads to vasocongestion of vagina, vulva, and clitoris; increased genital sensation; and vaginal lubrication and lengthening. Hormonal perturbations that alter any of these physiological processes may contribute to diminished genital sexual arousal.

Androgens have been shown to modulate the physiological function of many tissues and organs, including the hypothalamus, epididymis, adrenal gland, uterus, prostate, mammary gland, penis, thyroid gland, pituitary, levator ani muscle, kidney, vagina, clitoris, seminal vesicles, testis, liver, submaxillary gland, bulbocavernosus muscle, heart, and ovary. In addition, androgens regulate the development, growth, and maintenance of secondary sex characteristics (1).

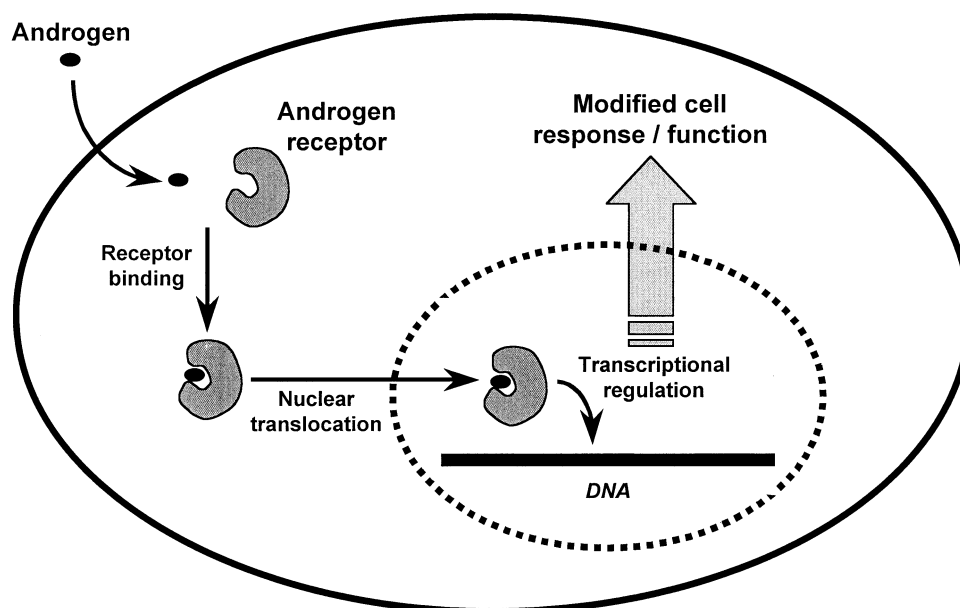
In the female, androgens are essential for the development of reproductive function and

are the immediate precursors for the synthesis of estrogens. Consequently, imbalance in androgen biosynthesis or metabolism may have undesirable effects on female general health and sexual reproductive function (2). The exact physiological and biochemical role of androgens in female genital sexual arousal function remains controversial and poorly understood. In postmenopausal women, Laan and van Lunsen (3) reported no association between sexual functioning and free T levels. It was suggested that androgens had no effect on sexual arousal per se but may influence aspects of sexual desire, such as thoughts and fantasies (4, 5).

However, other studies have suggested that androgens play an important role in female sexual arousal. Davis (6–10) and Davis and Tran (11) have suggested that androgen insufficiency in women is associated with impaired sexual function. Arlt et al. (12, 13) showed that DHEA replacement in women with adrenal

**FIGURE 1**

Mechanism of androgen action.



Traish. Androgen receptors in female genital tissues. *Fertil Steril* 2002.

insufficiency improves sexual function and well-being. Sherwin et al. (14) suggested that androgens are critical for maintenance of sexual functioning in postmenopausal women and that androgen may have a major impact on sexual desire. Myers and Morokoff (15) reported that serum testosterone (T) levels correlated with genital responses and subjective physical sensation (i.e., vaginal lubrication and breast sensation) in response to erotic visual stimulation.

These observations suggested that androgens play an important role in the physiology of genital arousal. Further, adequate genital sensation may be critical for maintaining sexual arousal and achieving orgasm. Sarrel (16, 17) suggested that hemodynamic events during genital sexual arousal are regulated by estrogens and enhanced by androgen supplementation. Vaginal lubrication is a combination of basal mucin production and vaginal vascular transudate. The vascular transudate constitutes the major lubrication component during genital arousal and is estrogen dependent. Mucin production and proliferation of vaginal epithelial cells are regulated by androgens (18–21). Future studies are needed to define the physiological and biochemical mechanisms by which androgens modulate female genital arousal.

### MOLECULAR AND CELLULAR MECHANISMS OF ANDROGEN ACTION

Androgen receptor (AR) expression is detected in varying degrees in many tissues. Androgens enter target and nontar-

get cells by passive diffusion. Once inside the target cell, biologically active androgens (T, 5 $\alpha$ -dihydrotestosterone) bind to a specific, soluble intracellular receptor protein molecule localized in the cytoplasm or in the nucleus (22–24). The binding energy of the hormone to its receptor results in physicochemical changes in the receptor complex, converting the receptor from a biologically inactive form to a biologically active state (22–24). These reactions initiated by hormone binding lead to interaction of the hormone receptor complex with unique and specific DNA enhancer elements referred to as *androgen response elements* (AREs) (25). The interaction of the activated AR complex with the AREs results in recruitment and binding of transcriptional factors, coactivators, or corepressors into the transcription complex and regulation of androgen-dependent gene transcription (26). A schematic representation of the overall biochemical mechanism of androgen action is depicted in (Fig. 1).

### ANDROGEN RECEPTOR STRUCTURAL AND FUNCTIONAL DOMAINS

The AR is a ligand-dependent nuclear transactivating factor and a member of the steroid–thyroid nuclear receptor superfamily (22–26). The members of this family are characterized by unique protein structure, which is comprised of distinct functional domains. These are [1] the hormone-binding domain, [2] the DNA-binding domain, [3] an amino terminal domain encompassing a transactivation function,

[4] the nuclear localization domain, and [5] the dimerization domain.

The AR is comprised of 918 amino acids, with an estimated molecular weight of 110 kDa (27–33). The hormone-binding domain is located near the carboxyl terminal region and is comprised of a hydrophobic region, which forms the hormone-binding site. The two predominant and naturally occurring androgens that bind to the AR are T and 5 $\alpha$ -dihydrotestosterone. *In vivo* and *in vitro* experiments have shown that 5 $\alpha$ -dihydrotestosterone binds more avidly to AR than T and is more potent in inducing biological response (34, 35). The higher potency of 5 $\alpha$ -dihydrotestosterone is attributed to the facts that 5 $\alpha$ -dihydrotestosterone binds to AR with greater affinity and dissociates more slowly and that the AR–5 $\alpha$ -dihydrotestosterone complex is more stable (36).

Recent studies have suggested that different genes may be activated by different ligands and that this may be reconciled by the presence of cell-specific and limiting transcription factors and coactivators. Also, the local concentration of the androgen hormone depends on the expression and activity of steroid-converting enzymes such as dehydrogenases and reductases. The latter enzymes play an important role in peripheral conversion of androgens to active or inactive metabolites. Also, the tissue- and cell-specific expression of different coactivators or corepressors in different target cells plays an important role in specific gene expression by androgens in various target tissues. The conformational changes in the hormone receptor complex are induced by ligand binding (T vs. 5 $\alpha$ -dihydrotestosterone). The energy of binding and the conformational changes in the protein are determinant for interaction with selective transcriptional factors, coactivators, and corepressors to be assembled in the transcription complex and determine which genes are regulated by androgens and therefore act as a discriminatory mechanism of gene activation.

The DNA-binding domain is comprised of 68 amino acids. This region is highly conserved among all members of this superfamily. The folding into tertiary structure of this region results in the formation of two distinct zinc fingers (formed by four cysteine residues noncovalently coordinating to the zinc ion within each finger) that bind to the DNA in the major groove. The first zinc finger confers specificity, and the second zinc finger contributes to increased affinity for binding to DNA (22–25). The AR binds to the palindromic ARE in a dimer form and in a cooperative manner. The dimer formation is suited to interaction with the ARE half-sites of the palindromic response elements.

Several other regions of the AR protein such as the ligand-binding domain and the loop of the second zinc finger contribute to stabilization of the dimer molecule (22–25). There is considerable homology between the DNA-binding domain of AR and that of progesterone, glucocorticoid, and mineralocorticoid receptors. Thus, regulation of gene expression by these proteins involves a complex mechanism

that requires interaction of the transactivation domains with other accessory factors such as transcriptional factors, coactivators, and corepressors (22–26)

The transactivation functional domain (TAF1) of the AR is localized in the amino terminal region (27–33). Unique sequences in this region are thought to interact with transcription factors and other accessory factors such as coactivators and corepressors (26–33). A second transactivation functional domain (TAF2) is localized to the hormone-binding region (29–33). Thus, regulation of gene expression by AR and the physiological response to androgens observed in various target tissues may be modulated by one or all of the following factors: [1] binding of a specific ligand (e.g., T vs. 5 $\alpha$ -dihydrotestosterone) to AR, [2] tissue-specific expression of AR, [3] differential binding of AR to AREs, or [4] tissue- and cell-specific expression of accessory transcriptional factors (coactivators and corepressors) necessary for interactions with the transactivation domain of the AR.

## ANDROGEN RECEPTOR EXPRESSION IN VAGINAL TISSUE

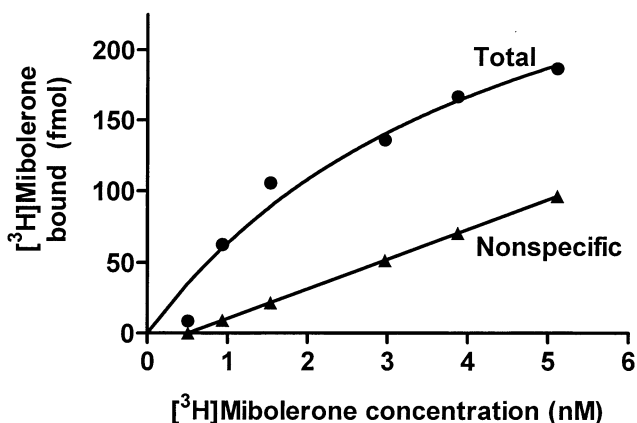
Immunohistochemical detection of androgen and estrogen receptors in vaginal tissues has been reported (37). However, limited biochemical and physiological data are available on the role of androgens in regulating female genital tissue structure and in modulating female genital sexual response. We determined AR concentrations in vaginal tissues from control and ovariectomized animals treated with estrogens or androgens.

We used radiolabeled 7 $\alpha$ , 17 $\alpha$ , dimethyl 19-nor-testosterone (mibolerone) as the ligand and 5 $\alpha$ -dihydrotestosterone as the competitive, nonradioactive ligand (38). This ligand binds AR specifically and with high affinity (Fig. 2). Specific protein-bound radioactivity, which is displaced by 5 $\alpha$ -dihydrotestosterone, represents bound ARs. Competitive binding analyses with vaginal tissue extracts showed that 5 $\alpha$ -dihydrotestosterone and T competed effectively for mibolerone binding, whereas DHEA and  $\Delta$ 5-androstenediol did not (Fig. 3). Because mibolerone binds to progesterone receptors, it is not surprising to note that progesterone and triamcinolone acetone also competed for binding of mibolerone.

We measured AR concentrations in extracts (cytoplasmic and nuclear) of proximal and distal vaginal tissues. Androgen receptor was expressed in proximal and distal vagina of intact animals (Fig. 4). Ovariectomy resulted in reduced AR levels in both proximal and distal vagina. Loss of AR expression in the proximal vagina of ovariectomized animals was restored when animals were treated with estrogens. Also, AR expression was enhanced in the distal vagina by treatment of ovariectomized animals with estrogens (Fig. 4).

**FIGURE 2**

Binding of [<sup>3</sup>H]mibolerone to vaginal tissue extract. Vaginal tissues from New Zealand White Rabbits were dissected and frozen on dry ice. Tissues were pulverized and homogenized as described elsewhere (38) in the presence of 0.4 M KCl to extract the nuclear receptor. The homogenate was centrifuged, and the supernatant (total tissue extract) was used for the binding assay. Aliquots of the extract were incubated with increasing [<sup>3</sup>H]mibolerone in the absence (total binding) or in the presence of 100-fold molar excess unlabeled 5 $\alpha$ -dihydrotestosterone (nonspecific binding). The samples were incubated for 48 hours at 0–2°C, and the bound radioactivity was separated by the hydroxylapatite assay method.



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## REGULATION OF VAGINAL NITRIC OXIDE SYNTHASE ACTIVITY BY ANDROGENS

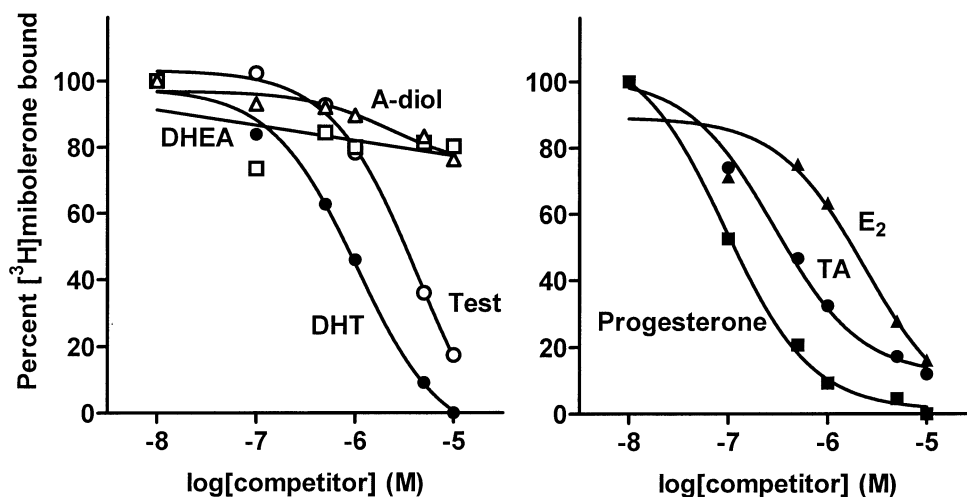
The physiologic and biochemical mechanisms of arousal regulating genital smooth muscle relaxation involve multiple signaling pathways. To date, several biochemical factors have been implicated. These include nitric oxide (NO), vasoactive intestinal polypeptide, and serotonin, among others (39). Studies from several laboratories have suggested that the NO–cyclic guanosine monophosphate (cGMP) pathway may play a role, among other pathways, in clitoral and vaginal smooth muscle relaxation (40). We have shown that smooth muscle cells cultured from human and rabbit clitoral and vaginal tissue expressed phosphodiesterase type 5 activity (41, 42).

We also demonstrated that treatment with NO donors increased cGMP accumulation in smooth muscle cells cultured from vaginal and clitoral tissue (41, 42). We have shown that pelvic nerve stimulation increased blood flow to the genitals and that the phosphodiesterase type 5 inhibitor, sildenafil, facilitated this response (43). Thus, we investigated the effects of estrogens and androgens on the expression and activity of vaginal nitric oxide synthase under various hormonal manipulations.

Nitric oxide synthase protein expression and activity were different in the proximal and distal regions of the vagina of control animals (44). We observed that ovariectomy in-

**FIGURE 3**

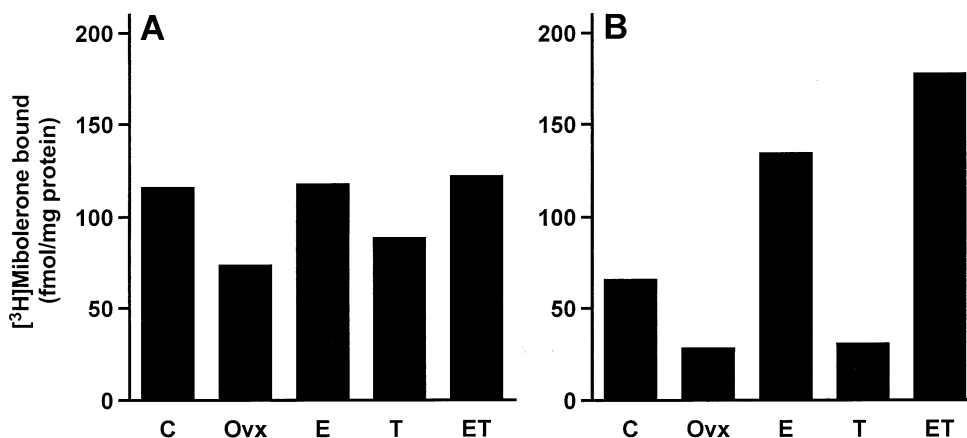
Displacement of [<sup>3</sup>H]mibolerone binding by unlabeled steroids. Aliquots of vaginal extracts were incubated at 0–2°C for 48 hours with 5 nM of [<sup>3</sup>H]mibolerone in the absence (total binding) or in the presence of increasing concentrations of unlabeled steroids. At the end of the incubation, the bound radioactivity was separated from free ligand by hydroxylapatite assay. Nonspecific binding was determined by incubation with [<sup>3</sup>H]mibolerone in the presence of 1000-fold excess unlabeled 5 $\alpha$ -dihydrotestosterone. DHT, dihydrotestosterone; A-diol,  $\Delta$ 5-androstenediol; triamcinolone acetonide, TA.



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**FIGURE 4**

Androgen receptor expression in proximal (A) and distal (B) vaginal tissues. Androgen receptor concentrations in vaginal tissue from control animals (C); ovariectomized animals (ovx); or ovariectomized animals treated with estradiol (E<sub>2</sub>), T, or a combination of E<sub>2</sub> plus T (ET) was determined by binding assay using [<sup>3</sup>H]mibolerone. Nonspecific binding was determined by competing the binding of [<sup>3</sup>H]mibolerone with 100-fold molar excess unlabeled 5 $\alpha$ -dihydrotestosterone. Only specific binding is represented.



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creased the expression of *n*-nitric oxide synthase in the proximal vagina, as assessed by Western blot analyses and enzymatic activity (Table 1) (44). Treatment of ovariectomized animals with T,  $\alpha$ -dihydrotestosterone, DHEA or  $\Delta$ 5 androstenediol resulted in marked increase in nitric oxide synthase expression and activity in the proximal vagina but not in the distal vagina (44). Treatment of ovariectomized animals with estradiol (E<sub>2</sub>) reduced nitric oxide synthase expression and activity in the proximal vagina. These observations suggest that androgens and estrogens differentially regulate nitric oxide synthase expression in the proximal region of the vagina. The implications of such observations remain to be determined.

### REGULATION OF ARGINASE ACTIVITY IN VAGINAL TISSUES BY ANDROGENS

When arginase activity in the proximal and distal vagina was determined, we observed that E<sub>2</sub> up-regulated arginase

activity in proximal vagina, whereas androgens down-regulated arginase activity in this tissue (Table 1) (44). Because L-arginine is a substrate for nitric oxide synthase, it is of interest to note that androgens decreased arginase activity while up-regulating nitric oxide synthase but that the reverse appeared to be the case with estrogen treatment.

### MODULATION OF VAGINAL SMOOTH MUSCLE CONTRACTILITY BY ANDROGENS

The contractile responses of vaginal strips from intact, ovariectomized and from ovariectomized, hormone-treated animals were investigated in organ bath studies. Androgen and estrogen deprivation and replacement had moderate effect on the contractile response to norepinephrine. Relaxation of precontracted vaginal strips to electric field stimulation was enhanced in animals treated with androgens and

**TABLE 1**

Summary of physiological responses and biochemical data obtained from ovariectomized animals treated with androgens or estrogens.

Physiological response	Method	Estrogen	Androgen
Vaginal muscle relaxation	Organ bath	Attenuated	Potentiated
Estrogen receptor	Radioligand binding and Western blot	Decreased	Increased
Nitric oxide synthase	Enzyme activity and Western blot	Decreased	Increased
Arginase	Enzyme activity	Increased	Decreased

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was attenuated in tissues from animals treated with estrogens. Similarly, vaginal strips precontracted with norepinephrine relaxed in a dose-dependent manner to increasing concentrations of vasoactive intestinal polypeptide. The relaxation was enhanced in tissues from ovariectomized animals treated with T, 5 $\alpha$ -dihydrotestosterone,  $\Delta$ 5 androstenediol, or dehydroepiandrosterone (Table 1). In contrast, relaxation was attenuated in tissues from ovariectomized animals treated with E<sub>2</sub> alone or with E<sub>2</sub> and T or with E<sub>2</sub> plus progesterone.

These observations suggested that androgens enhance the relaxation of the vaginal smooth muscle to endogenous nonadrenergic, noncholinergic released neurotransmitters and to exogenous vasoactive intestinal polypeptide, whereas E<sub>2</sub> attenuated these responses.

## DISCUSSION

Although androgens are known to influence the physiology of clitoris, labia, and vagina, the role of androgens in female sexual function and dysfunction and in particular to female genital sexual arousal response remains controversial and poorly understood. Women undergoing androgen therapy to alleviate symptoms associated with menopause reported increased sexual interest and heightened desire. Greenblatt et al. (45) and Salmon and Geist (46) have shown that androgen treatment dramatically increased sexual interest and orgasmic responses and increased sexual satisfaction. Whether the effects of androgens are mediated by direct action on the nervous system, by effect on the genital organs, or both has not been determined. Although the results of several clinical studies have pointed to the potential usefulness of androgens in treating female sexual dysfunction (6–13, 16, 17, 47), it remains unclear how androgens modulate vaginal and clitoral physiology.

Physiologic study of the arousal phase of the female sexual response involves, in part, an understanding of the various local regulatory mechanisms, which modulate tone in the clitoral erectile tissue and the vaginal nonvascular smooth muscle (48–51). The role of estrogens in the maintenance and function of female genitalia has been investigated (3, 16, 17, 51–54). Thickness and rugae of the vaginal wall, as well as vaginal lubrication, have been shown to be estrogen dependent. Although vaginal lubricant production has been shown to be hormone dependent, both in the resting state and during sexual excitement, quantitative changes apparently do not occur during the menstrual cycle (51).

Estrogens enhance and improve genital sensation and also maintain blood flow (3, 16, 17). Estrogen replacement therapy increases pelvic blood flow in menopausal women and in women with surgical or medical ovariectomy. Estrogen deficiency results in thin vaginal walls more easily susceptible to trauma and with a decreased ability to heal, as well as in a drier and less acidic vaginal environment more

vulnerable to infection (54). Vaginal dryness is associated with ovarian failure and is effectively controlled by estrogen replacement therapy (3, 52). Estrogens may affect smooth muscle cell growth in the vagina and the clitoris, may regulate connective tissue metabolism and nitric oxide synthesis, and may be important in maintaining the functional integrity of vaginal and clitoral smooth muscle function.

Regulation of vaginal and clitoral physiology by androgens is an area of great interest but has yet to be investigated. Our studies have shown that vaginal tissues express ARs and that the expression of these receptors is regulated differently in the proximal and distal vagina by androgens and estrogens. Further, we have observed that relaxation of nonvascular vaginal smooth muscle is facilitated by androgen priming and is attenuated by estrogens. These observations are also supported by increased synthesis and activity of nitric oxide synthase in the proximal vaginal tissues in response to androgens, whereas nitric oxide synthase synthesis and activity decreased in response to estrogens.

Clearly, androgens appear to play a role in vaginal and clitoral function during genital sexual arousal. Specific AR proteins mediate the physiological responses to androgens in many tissues. It is believed that the physiologic response in vaginal tissues to androgens is also mediated by specific ARs. The effects of androgens may be related to maintenance of nonvascular smooth muscle function in the vagina and of vascular smooth muscle in the clitoris. It remains unclear; however, which androgen metabolites are responsible for maintaining tissue structure and function. This is an area of great scientific and clinical interest and will be a focus of future research.

The physiological role of androgens in women is confounded by the fact that synthesis and metabolism of androgens take place in the ovaries, adrenal gland, and peripheral tissues (2, 55–57). This suggests complex regulation of synthesis of androgens by the various organs and tissues and the enzymes involved in biotransformation. Testosterone and dihydrotestosterone are the active androgens; however, androgen metabolites such as  $\Delta$ 5 androstenediol also possess androgenic activity (58). Further, active androgens can be synthesized in the target tissue on demand; thus, levels of plasma T may not provide the critical information on the availability of other metabolites. Biochemical alteration in androgen biosynthesis in the ovary, adrenal, or peripheral tissues may lead to androgen insufficiency.

Reports of premenopausal women with adequate estrogen levels but low androgen levels suggest potential metabolic alteration in androgen biosynthesis. Thus, aromatization of androstenedione, the major androgen produced by the ovary, to estrone and E<sub>2</sub> brings about sufficient estrogen levels, but its conversion to T may be limited, resulting in low levels of T in plasma. Similarly, adrenal insufficiency may result in low synthesis of androgens. Thus, androgen insufficiency in

women may have multiple etiologies and requires further investigation.

Furthermore, the peripheral conversion of androgens (55–57) in many tissues, including genital tissues, suggests that different androgen hormones may act either via the classical AR or through putative AR proteins. It is therefore important to delineate the role of androgen-binding proteins in vaginal and clitoral tissues and how androgens influence the biochemical and physiological function of the genital tissues and in turn female sexual arousal. We suggest that local tissue concentration of the steroid hormone, which may be dependent on local peripheral conversion, is important in mediating the tissue-specific physiological response. Also, the local concentration of androgen and estrogen and the ratio between these hormones in genital tissue may be important in eliciting the physiological response. Finally, tissue-specific enzymes such as nitric oxide synthase and arginase and receptor proteins appear to be regulated differentially by steroid hormones.

In summary, we have characterized androgen expression in proximal and distal vaginal tissues from control and ovariectomized animals treated with or without estrogen replacement therapy and/or androgen replacement therapy. We demonstrate that androgens enhance nitric oxide synthase expression and activity and down-regulate arginase activity in the proximal vagina. This biochemical change may be manifested in facilitation of vaginal smooth muscle relaxation to electric field stimulation and vasoactive intestinal polypeptide in androgen-treated animals. Estrogens, however, down-regulate nitric oxide synthase activity and increase arginase activity. This may result in attenuation of vaginal tissue relaxation to electric field stimulation and to vasoactive intestinal polypeptide. These observations suggest that androgens play an important role in modulating the physiology of vaginal tissue and may contribute to modulation of the genital sexual arousal response in women.

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