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Differential Effects of Testosterone and Estradiol on Clitoral Function: An Experimental Study in Rats

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ABSTRACT

Introduction: Female sexual response is a complex phenomenon in which psychological, neurologic, and vascular mechanisms and hormonal factors interact. During the arousal phase, they cooperate to increase genital blood flow, thus inducing engorgement of the clitoris and lubrication of the vagina. Regulation of vascular and non-vascular smooth muscle tone is the crucial event in the erectile process. Preclinical studies have suggested that nitric oxide (NO) is the main vasodilator neurotransmitter modulating, through the second messenger cyclic guanosine monophosphate (cGMP), clitoral flow vessels.

Aim: To investigate the effects of sexual steroid hormones on pro-erectile and relaxant (mediated by NO and cGMP) and anti-erectile and contractile (mediated by ras homolog gene family member A [RhoA] and Rho-associated protein kinase [ROCK]) mechanisms in the clitoris using a validated animal model of female ovariectomized Sprague-Dawley rats.

Methods: Subgroups of ovariectomized rats were treated with 17β-estradiol, progesterone, testosterone, or testosterone and letrozole for 6 weeks. The experimental groups were compared with a control group of intact rats.

Main Outcome Measures: Sex steroids plasma levels were assessed and *in vitro* contractility studies were carried out in order to investigate the effect of ovariectomy and *in vivo* treatments on clitoris smooth muscle activity. Smooth muscle cells (SMCs) from rat clitoral biopsies were isolated and characterized. RhoA activity was determined in SMCs cell cultures. RNA from tissues and cells was analyzed by quantitative real-time RT-PCR.

Results: Using real-time polymerase chain reaction, testosterone treatment upregulated the expression of NO-mediated pathway genes (endothelial and neuronal NO synthase, guanylate cyclase soluble subunit-α_3, guanylate cyclase soluble subunit-β_3, cGMP-dependent protein kinase 1, and phosphodiesterase type 5). Conversely, estrogen replacement upregulated the expression of calcium-sensitizing RhoA-ROCK pathway genes. In vitro contractility studies were performed on phenylephrine pre-contracted clitoris strips. Ovariectomy resulted in a decreased responsiveness to Y-27632, a ROCK inhibitor, which was fully restored by 17β-estradiol supplementation. To further examine the effect of 17β-estradiol on the RhoA-ROCK pathway, smooth muscle cells were isolated from rat clitoris and their migration capacity was evaluated.

Conclusion: Collectively, these data demonstrate that testosterone improves the relaxation of vascular smooth muscle cells through the NO-cGMP pathway, and that testosterone and 17β-estradiol are necessary to maintain a functional contractile and relaxant machinery in the clitoris. This new concept might provide support for the concomitant use of estrogen and testosterone during the treatment of sexual arousal disorders related to hormonal imbalance or insufficiency.

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INTRODUCTION

Female genital sexual arousal is elicited by sensory stimulation and by activation of multiple brain areas, resulting in increased blood flow to the genitals. Among genital vascular compartments, those composed of erectile tissues, namely the clitoris and clitoral bulbs, demonstrate the greatest vasocongestion and volume change during sexual arousal.[1]

After sexual stimulation, the decrease of central sympathetic tone and the release of vasodilator neurotransmitters (such as vasoactive intestinal peptide and nitric oxide [NO]) rapidly increase blood flow to the clitoris, which becomes fully vasocongested and tumescent. NO stimulates guanylyl cyclases (GCs), which convert guanosine-5′-triphosphate (GTP) to cyclic guanosine monophosphate (cGMP). The latter induces smooth muscle relaxation by multiple mechanisms, including stimulation of a cGMP-dependent protein kinase (PKG).[2−5] In addition to GCs, intracellular cGMP levels are finely regulated by phosphodiesterase type 5 (PDE5), the most important player in cGMP cleavage and degradation. PDE5 expression[6−8] and activity[9] have been found in the corpus cavernous of the human clitoris and vagina. In the basal, non-stimulated condition, a high vasomotor tone of the arterial supply, through central sympathetic activation, keeps clitoral blood flow at the minimal (ie, resting) level.[1] Ras homolog gene family member A (RhoA) and Rho-associated protein kinase (ROCK) signaling, one of the most well-characterized mechanisms upregulating smooth muscle tone in male genitalia,[10] has been suggested to mediate smooth muscle contraction in the clitoral corpus cavernosum.[11] Nonetheless, large gaps exist in our knowledge concerning the physiology of female genital arousal and particularly the molecular mechanisms involved in clitoral smooth muscle contraction, even if some extrapolations from the male counterpart can be assumed.

Adding layers of complexity, changes in the sex steroid milieu could modulate the female response during genital arousal. It is widely accepted that menopause-associated estrogen decrease is responsible for decreased pelvic blood flow, causing vaginal dryness and hypo-lubrication.[12,13] Compelling evidence indicates that administration of vaginal estrogens is an effective intervention for menopausal-related vaginal dryness and atrophy.[14] In addition to estrogens, testosterone (T) can modulate the female genital sexual arousal response. A recent systematic review and meta-analysis of randomized controlled trials on the effects of systemic T therapy in postmenopausal women demonstrated that the use of T alone or in combination with hormonal replacement therapy (HRT) significantly improved multiple domains of sexual functioning, including the arousal domain.[15] However, the molecular mechanisms underlying the physiologic and pharmacologic actions of estrogens and T in genital sexual arousal have not been completely unraveled. Moreover, although the effects of sex steroids on the vagina have been well established in experimental models,[1] their role on clitoral function is not well understood and requires further investigation.

AIMS

The aim of the present study was to investigate the effect of the sex steroid milieu on pro-erectile and relaxant (NO-cGMP) and anti-erectile and contractile (PDE5 and RhoA-ROCK) pathways in the clitoris. For this purpose, we used a previously established animal model of ovariectomized (OVX) female rats, which were treated with estradiol (E2), progesterone (P), T, or T and letrozole (L), to completely abrogate T-induced estrogen formation.

METHODS

Animals

Sprague-Dawley rats (Envigo, San Pietro al Natisone, Udine, Italy) were individually caged under standard conditions in a temperature- and humidity-controlled room on a 12-hour light and dark cycle. Water and food were unrestricted throughout the study until sacrifice by cervical dislocation. Corpora cavernosa were harvested from a group of male Sprague-Dawley rats (235−260 g; n = 11) for sex-comparison studies. In addition, 146 mature female Sprague-Dawley rats (235−260 g) were randomly assigned to an intact group (control, n = 54) and an OVX group (n = 92). Female rats were bilaterally ovariectomized under chloral hydrate anesthesia (1 mL/100 g of body weight of a 3.6% solution, injected intraperitoneally). After anesthesia, the rat was placed in ventral recumbency with the tail toward the surgeon. The dorsal midlumbar area was shaved and swabbed with surgical scrub, iodine, and alcohol. A 2- to 3-cm dorsal midline skin incision was made halfway between the caudal edge of the ribcage and the base of the tail. A single incision 5.5 to 10 mm long was made into the muscle wall on the right and left sides approximately one third the distance between the spinal cord and the ventral midline. The ovary and oviduct were exteriorized through the muscle wall. A hemostat was clamped around the uterine vasculature between the oviduct and uterus and each ovary and part of the oviduct was removed with single cuts through the oviducts near the ovary. The hemostat was removed and the remaining tissue was replaced into the peritoneal cavity. The ovary on the other side was removed in a similar manner and the muscle incision was not sutured. A group of OVX rats did not receive any treatment (n = 25), whereas 2 weeks after ovariectomy hormonal treatment was started in four subgroups of OVX rats. One subgroup was supplemented with intramuscular injections of T (30 mg/kg weekly; OVX + T group, n = 22). Another subgroup was treated with