Combination of Testosterone and Vardenafil Increases Female Sexual Functioning in Sub-Primed Rats

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ABSTRACT

Introduction. Hypoactive sexual desire disorder (HSDD) is a common problem in women and may have a negative impact on quality of life. A recent clinical study shows an increase in sexual drive of HSDD women after cotreatment of testosterone and vardenafil (phosphodiesterase type 5 inhibitor).

Aim. In this study, we investigated the effect of testosterone and vardenafil on sexual activity in female rats.

Main Outcome Measures. Proceptive (darts and hops), receptive (lordosis), and paced-mating (percentages after exits and contact-return latencies) behaviors were quantified.

Methods. Ovariectomized female rats, sub-primed with only estradiol and fully primed with estradiol and progesterone, were tested in a paced-mating sex test and sexual behaviors were quantified. The sub-primed rats are thought to model HSDD. The effect of testosterone (100 and 300 μg, subcutaneous [SC]) and vardenafil (10 mg/kg, per os [PO]) alone and testosterone (300 μg, SC) in combination with vardenafil (3 and 10 mg/kg, PO) were tested. We also studied the effects of testosterone (300 μg, SC) + intracerebroventricular (ICV) injections of vardenafil (25 and 50 μg) on sexual activity.

Results. No effect of testosterone and vardenafil alone was found, but cotreatment of testosterone and vardenafil (PO) caused a significant increase in proceptive and receptive behavior in the sub-primed female rats. Testosterone and vardenafil did not affect fully primed females. ICV administration of vardenafil combined with systemic testosterone, on the other hand, had no effect on sexual activity in both sub-primed and fully primed female rats.

Conclusions. We conclude that cotreatment of subcutaneous testosterone and oral vardenafil increase sexual activity in sub-primed female rats. Our data supports the human finding that combination treatment of testosterone and vardenafil could be used as a new treatment for women with HSDD. **Snoeren EMS, Bovens A, Refsgaard LK, Westphal KGC, Waldinger MD, Olivier B, and Oosting RS. Combination of testosterone and vardenafil increases female sexual functioning in sub-primed rats. J Sex Med 2011;8:989–1001.**

Key Words. Sexual Behavior; Female Rat; Hypoactive Sexual Desire Disorder; PDE-5 Inhibitor; Animal Model; Testosterone

Introduction

According to the Diagnostic and Statistical Manual of Mental Disorders, 4th edition [1], female sexual dysfunction (FSD) can be divided in four main categories: low sexual desire, low arousal, orgasmic disorders, and sexual pain. Each is defined as "persistent or recurrent" and causes "marked distress or interpersonal difficulty." The prevalence of FSD in the human population ranges from 33–48% in the United States and in Europe [2–8]. The majority of sexual dysfunction surveys identify low sexual desire/interest (also called hypoactive sexual desire disorder, HSDD) and sexual arousal disorder as the most common problems [4–8].

The biological mechanisms underlying the different types of FSD are not well known, and may differ between women. Especially, decreased libido and lack of sexual arousal may be due to hormonal changes. The menopause and ovariectomy cause a drop in hormone levels and may lead to FSD [9,10]. Ovariectomy causes both a drop in estradiol and testosterone [11,12], while the natural menopause only affects estradiol levels without affecting circulating testosterone levels [13]. A few double-blind studies have shown that hormone replacement therapy with estrogens could have beneficial effects on sexuality in women with hormone deficiencies [14,15]. Sometimes, testosterone is administered together with estrogens in ovariectomized women and improve sexual function and decrease distress [16]. Unfortunately, this effect is only seen in these specific group of patients and not in other forms of FSD [17].

For men with erectile dysfunctions, phosphodiesterase type 5 (PDE-5) inhibitors, like sildenafil and vardenafil, are effective treatments [18,19]. Whether these medications are beneficial in women with sexual disorders is unclear. Some studies showed an increase in clitoral sensitivity [20], arousal, and frequency of sexual fantasies, sexual intercourse, and orgasm [21]. There is only one randomized and placebo-controlled clinical trial performed and this trail showed no improvement of vardenafil on sexual response among women with sexual arousal disorder [22]. Overall, no clear proof of an effect of PDE-5 inhibitors on female sexual functioning is available.

Recently Van der Made et al. [23] showed positive effects of testosterone combined with vardenafil on females with low sexual desire disorders. They showed that the combination of testosterone and vardenafil enhanced their sexual motivation during exposure to erotic visual stimuli. This effect was only seen in females suffering from HSDD and not in healthy women. Thereby, this research offers an interesting potential treatment for women suffering from HSDD.

As animal model of HSDD, we used the so-called "sub-primed" model in which female rats are ovariectomized and administered low doses of estradiol. As comparison, "fully primed" rats were injected with both estradiol and progesterone. The optimal hormone doses to obtain sub- and fully primed females were first determined.

Based on the study of Van der Made et al. [23], we performed an experiment in which we investigated the effect of testosterone and vardenafil alone and combined in sub- and fully primed rats. We found that the combined treatment increased sexual excitement in the sub-primed female rats. Next, we investigated whether this stimulatory effect was due to a central or peripheral action of vardenafil. In all experiments, we measured the number of proceptive (darts and hops), receptive (lordosis quotient and lordosis score), and paced-mating (percentage of exits and contact-return latency) behaviors.

Materials and Methods

Animals

For the first three experiments, Wistar female rats (N = 45, 3 months of age at the beginning of the experiment) and stimulus Wistar male rats (N = 45, 6 months of age) were used (Harlan, Zeist, the Netherlands). In the last experiment, new females and males (both N = 45, 3 months of age) were used. All animals were housed in the Central Animal Laboratory of the Utrecht University. The rats were adapted to the laboratory environmental condition and a reversed 12/12-hour light-dark cycle (lights off at 7 AM). Standard food and water were available ad libitum. Furthermore, male and female rats were housed in separate rooms in groups of four per Macrolon type IV in home cage ($60 \times 38 \times 20$ cm). The male rats were sexually trained with a different set of females before the experiments.

The female rats were bilaterally ovariectomized under isoflurane anesthesia 14 days before the start of the experiment. Sexual receptivity was induced by subcutaneous administration of estradiol benzoate (2 or 5 μg EB) alone or 5 μg estradiol in combination with progesterone (500 μg P). The hormones were dissolved in 0.1 mL sesame oil saturated with phosphatidylcholine 36 (EB) and 4 (P) hours prior to testing. The combination of estradiol and progesterone (fully priming) induces full receptive and proceptive behavior in females, whereas a single injection with estradiol (sub-priming) induces low levels of receptivity. The sub-priming females could therefore model female sexual dysfunction.

All experiments were carried out in accordance with institutional, national, and international guidelines for animal care and the Dutch law concerning welfare.

Drugs

Testosterone propionate (Spruyt-hillen, IJsselstein, the Netherlands) was dissolved in 0.1 mL