TESTOSTERONE REPLACEMENT THERAPY IMPROVES VOIDING DYSFUNCTION IN OVARIECTOMIZED (OVX) RATS

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HYPOTHESIS / AIM OF STUDY

Menopause reduces the levels of sexual hormones. Urological complications are commonly observed in the postmenopausal women (1). The urinary symptoms associated with late postmenopause include frequency, nocturia, urgency and urge incontinence. Testosterone replacement therapy has favorable effects on the lack of sexual desire, bone loss and decreased muscle mass (2, 3). However, little is known about the effects of testosterone replacement in the urinary symptoms in postmenopause. Therefore, in the present study, we used a rat model of ovariectomy to evaluate the effects of testosterone in voiding dysfunction and *in vitro* bladder alterations.

STUDY DESING, MATERIALS AND METHODS

- Two-month old female Sprague–Dawley rats (250-280 g) were anesthetized (ketamine/xylazine, 60:6 mg/kg, IP), and submitted to bilateral ovary removal, whereas sham-operated rats were manipulated but ovaries were left intact. Rats were divided into the following groups: Sham, OVX and OVX plus testosterone undecanoate treatment (IM).
- After 30 days of treatment, the general characteristics of ovariectomy model (body, perigonadal fat mass, uterus, bladder and urethra weights), as well as the arterial pressure by tail-cuff pressure technique were evaluated. Blood samples were collected for testosterone serum quantification by chemmiluminescence.
- Urodynamic studies were realized in anaesthetized animals (urethane 1.8 g/kg) by cystometry.
- A separate group had the bladder and urethra removed. *In vitro* functional assays were evaluated in isolated bladder strips using a 4 mL myograph system and isolated urethra ring using 5 mL myograph system, each system containing Krebs solution (*p*H 7.4, 37 ºC, O_2/CO_2 (95%: 5%)). In the bladder, concentration-response curves for the muscarinic agonist carbachol (CCh) and the β_3–selective agonist mirabegron were conducted. Electrical Field Stimulation (EFS) was applied in bladder tissue placed between two platinum electrodes connected to a Grass S88 stimulator at 80 V, 1 ms pulse width and trains of stimuli lasting 10 s at varying frequencies (1-32 Hz). In the urethra, concentration-response curves for angiotensin II (Ang II) were conducted.

RESULTS

Bilateral ovariectomy significantly increased body weight, blood pressure and perigonadal fat mass, reduced uterus weight and diminished the hormonal levels of testosterone compared with Sham group.

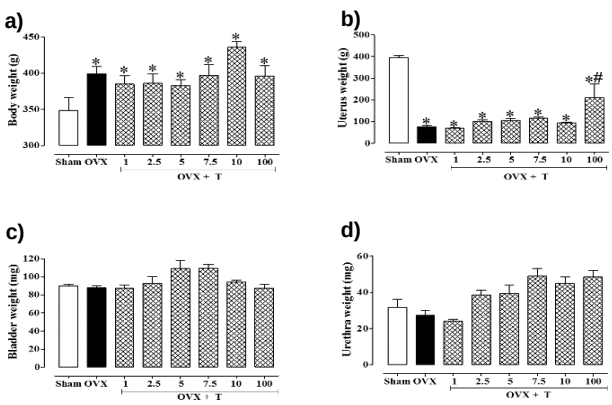

Fig 1. Body (a), uterus (b), bladder (c) and urethra weight (d) of Sham and OVX rat treated or not with testosterone in different doses (1-100 mg/kg). Data represent the mean ± SEM, n = 5 animals/group. *P <0.05 compared with Sham group; (One-way ANOVA followed by Bonferroni test).

Table 1. Perigonadal fat and systolic blood pressure (SBP) of Sham and OVX groups treated or not with testosterone (5 mg/kg and 10 mg/kg)

Groups	Perigonadal fat (g)	Blood pressure (mmHg)
Sham	5.5 ± 0.4	108 ± 8.5
OVX + Vehicle	8.7 ± 0.4*	141 ± 3.8*
OVX + T (5 mg/kg)	8.8 ± 1.4*	140 ± 4.5*
OVX + T (10 mg/kg)	9.0 ± 0.6*	142 ± 4.2*

Data are represent means ± SEM, 5 animals/group. *P < 0.05 vs compared with Sham group; (One-way ANOVA followed by Bonferroni teste).

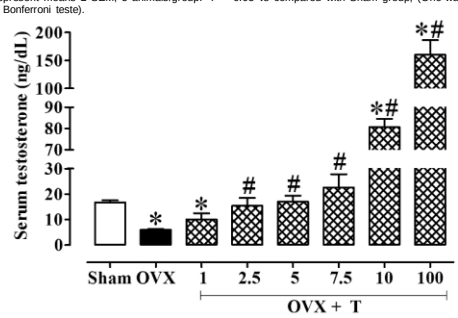

Fig 2. Testosterone serum level of Sham and OVX rat treated or not with testosterone in different doses (1-100 mg/kg). Data represent the mean ± SEM, n = 5/group. *P <0.05 compared with Sham group; (One-way ANOVA followed by Bonferroni test).

The voiding dysfunctions in OVX rats were characterized by increases of basal pressure, threshold pressure, voiding frequency and post-micturition pressure.

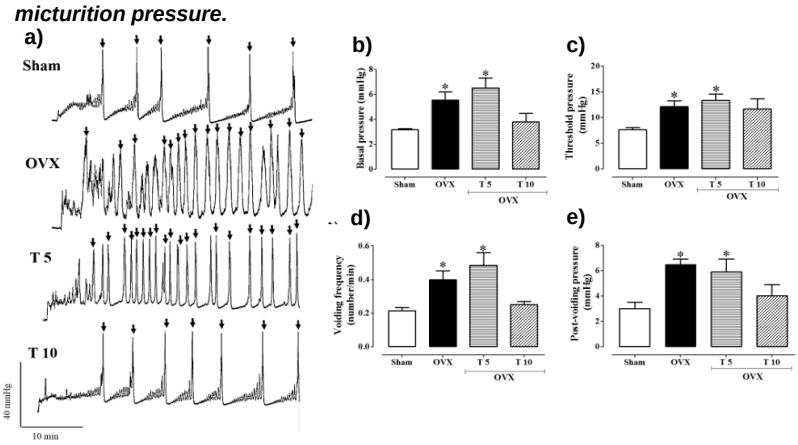

Fig 3. Cystometric evaluation in anesthetized ovariectomized (OVX) rats treated or not with testosterone at physiological (5 mg/kg) or supraphysiological (10 mg/kg) levels in comparison with Sham group. Panel A shows the representative cystometric traces. Panels B to E shows basal pressure, threshold pressure, voiding frequency and post-voiding pressure, respectively. Data represent the mean values ± SEM (n = 5-12 animals/group). *P < 0.05 compared with Sham group.(One-way ANOVA followed by Bonferroni test).

In vitro bladder contraction to either carbachol or EFS (1-32 Hz) were significantly reduced, whereas Ang II-induced urethral contractions were increased in OVX compared with Sham group. No significant changes in the bladder relaxant response between OVX and Sham group.

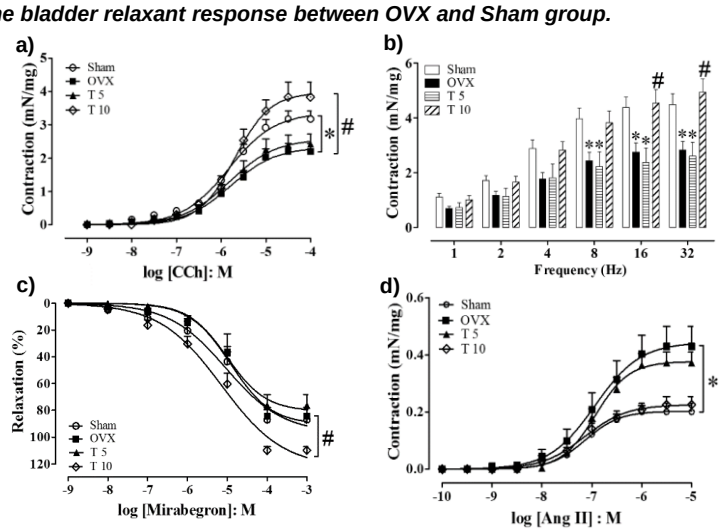

Fig 4. *In vitro* bladder and urethra contractions and relaxations. Curve concentration-response to carbachol (CCh) (a), contraction to electrical-field stimulation (1-32 Hz) (b), curve concentration-response to mirabegron (c) and curve concentration-response to angiotensin II (Ang II) (d) of ovariectomized (OVX) rats treated or not with testosterone at physiological (5 mg/kg) or supraphysiological (10 mg/kg) levels in comparison with Sham group. Data represent the mean ± SEM, n = 6-10 animals/group. *P <0.05 compared with Sham group; #P <0.05 compared with OVX group (One-way ANOVA followed by Bonferroni test).

INTERPRETATION OF RESULTS

According to our study, supraphysiological dose of testosterone (10 mg/kg) improves the voiding dysfunctions and the *in vitro* alterations of bladder and urethra smooth muscle. It is likely that testosterone acts by activating the androgen receptor in the lower urinary tract, triggering genomic and non-genomic signaling pathways. However, additional studies are required to elucidate these mechanisms.

CONCLUSION

Testosterone replacement at a physiological dose (5 mg/kg) does not improve the bladder dysfunctions by ovariectomy; however, at a supraphysiological dose (10 mg/kg), testosterone replacement normalizes all the functional changes in lower urinary tract smooth muscle. Our findings suggest that androgen therapy may have therapeutic benefits for urologic complications associated with postmenopause.

REFERENCES

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Disclosures: Ethical Principles in Animal Research adopted by the Brazilian College for Animal Experimentation and approved by the Institutional Committee for Ethics in Animal Research of State University of Campinas (Protocol No 3500-1)

Financial suport: CAPES CNPq FAPESP