Testosterone and estradiol mediate male voiding dysfunction by reducing prostatic smooth muscle Ppp112b abundance and impairing muscle relaxation

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Background

- Lower urinary tract symptoms (LUTS) are benign urinary symptoms such as straining to urinate, weak stream, and increased urinary frequency, especially at night.
- Lower urinary tract symptoms are prevalent in men of advancing age and often correlated with changes in testosterone (T) and estradiol (E2) levels.
- Drugs that smooth muscle relaxation are commonly prescribed in the clinic, but it is unknown why some men have hypertension prostatic smooth muscle.
- Here, we examine whether exogenous testosterone and estradiol influence smooth muscle contraction or relaxation dynamics leading to urothelial obstruction.

Hypothesis

T+E2 reduces Ppp112b abundance, promoting tonic lower urinary tract smooth muscle contractions and priming bladder outflow obstruction

Materials and Methods

**T+E2 Implants**

- Male C57BL/6 mice were administered subcutaneous compressed pellets of 20 mg testosterone + 1.5 mg estradiol. After 2 weeks, mice were sacrificed using the following:
  - **Validator Array**
    - Measure mouse urine pattern and number during a 24-hour observation period
    - Hypothesis: Frequency of voiding (urinary output) will be increased by T+E2 treatment.
  - **Contrast Enhanced Ultrasound**
    - Measure velocity of contrast through the mouse prostatic urethra
    - Hypothesis: Velocity of contrast will be increased by T+E2 treatment due to prostatic smooth muscle contraction.

- **Smooth Muscle Physiology**
  - Measure prostatic smooth muscle contraction and relaxation metrics using changes in fluorescence via GCAMP5 mouse and tissue bath
  - Hypothesis: T+E2 treatment increases duration of smooth muscle relaxation

- **RT-PCR and Protein Analysis**
  - Measure mRNA and protein levels of myosin phosphatase subunit 2-like (MYPH2) in mice of prostatic urethra
  - Hypothesis: T+E2 treatment decreases levels of the myosin phosphatase to impair prostatic smooth muscle relaxation.

- **Genetic depletion of Ppp112b**
  - Mice with genetic deletion of Ppp112b partially phenocopy T+E2 treatment by causing delays in prostatic smooth muscle relaxation.

Results

- **T+E2** reduces voiding dysfunction in mice by increasing urinary frequency, total urinal output, and changing voiding behavior.
  - Before T+E2: 2 weeks T+E2
- **T+E2** implants increase the magnitude and sustain the ureodynamic response of alpha-1 adrenoceptor agonist phenylephrine.

Conclusions

- T+E2 increases urinary voiding frequency, total urinal output, and percent urinal output in the bladder void spot assay.
- An alpha-1 adrenoceptor agonist (phenylephrine) increases the magnitude and sustains ureodynamic response in mouse prostatic urethra.
- T+E2 prolongs prostatic smooth muscle relaxation after phenylephrine administration via GCAMP and tissue bath analysis.
- T+E2 downregulates myosin phosphatase subunit Ppp112b mRNA and protein in prostate.
- Genetic depletion of Ppp112b partially phenocopies T+E2 by delaying prostatic smooth muscle relaxation.

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