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 correlate 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 hypercontracted prostate smooth muscle.
- Here, we examine whether exogenous testosterone and estradiol influence smooth muscle contraction or relaxation dynamics leading to urethral obstruction.

Hypothesis

T+E2 reduces *Ppp1r12b* abundance, promoting tonic lower urinary tract smooth muscle contractions and priming bladder outflow obstruction

Materials and Methods

T+E2 dosing

C57Bl/6J mice were administered subcutaneous compressed pellets of 25 mg testosterone + 2.5 mg estradiol. After 2 weeks, mice were assessed using the following:

Void Spot Assay

Measure mouse void pattern and number during a 4-hour observation period
Hypothesis: Frequency of small volume (droplet voids, indicative of bladder outlet obstruction) will be increased by T+E2 treatment.

Contrast Enhanced Ultrasound

Measure velocity of contrast passage through the mouse prostatic urethra
Hypothesis: velocity of contrast passage will be increased by T+E2 treatment caused by prostatic smooth muscle obstruction.

Smooth Muscle Physiology

Measure prostate smooth muscle contraction and relaxation metrics using changes in fluorescence via GCaMP mice and tissue bath
Hypothesis: T+E2 treatment increases the duration of smooth muscle relaxation

mRNA and Protein Analysis

Measure mRNA and protein levels of myosin phosphatase subunits
Hypothesis: T+E2 exposure downregulates a subunit of the myosin phosphatase to impair prostate smooth muscle relaxation

Genetic depletion of Ppp1r12b

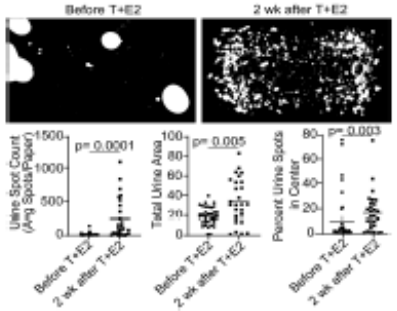
Use mice with a genetic depletion of Ppp1r12b to analyze prostate smooth muscle dynamics
Hypothesis: Mice with genetic depletion of Ppp1r12b partially phenocopy T+E2 treatment by causing delayed prostate smooth muscle relaxation

Conclusions

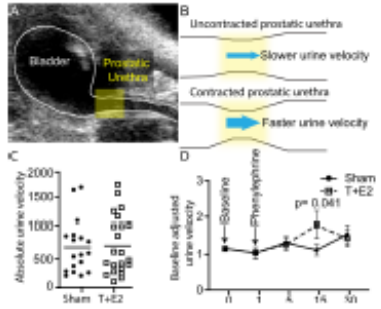
- T+E2 increases urinary voiding frequency, total urine area, and percent urine spots in the center via void spot assay
- An alpha 1 adrenoreceptor agonist (phenylephrine) increases the magnitude and sustains urodynamic response in mouse prostatic urethra
- T+E2 prolongs prostate smooth muscle relaxation after phenylephrine administration via GCaMP and tissue bath analysis
- T+E2 downregulates myosin phosphatase subunit PPP1R12B mRNA and protein in prostate
- Genetic depletion of PPP1R12B partially phenocopies T+E2 by delaying prostate smooth muscle relaxation.

Results

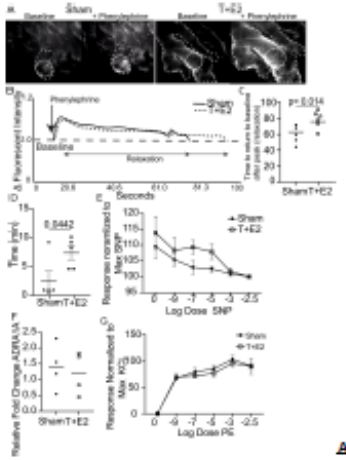
T+E2 drives voiding dysfunction in mice by increasing urinary frequency, total urine area, and changing voiding behavior



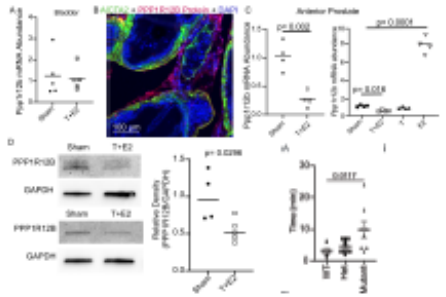
T+E2 implants increase the magnitude and sustain the urodynamic response of alpha-1 adrenoceptor agonist phenylephrine



T+E2 prolong prostate smooth muscle relaxation after alpha-1 adrenoceptor mediated contraction via GCaMP and tissue bath analysis



T+E2 significantly decreases PPP1R12B mRNA and protein abundance. Genetic depletion of PPP1R12B delays prostate smooth muscle relaxation via tissue bath



Acknowledgements: This study was supported by NIH grants F31ES030968, U54DK104310, and RO1ES001332