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EFFECTS OF LOW TESTOSTERONE AND TESTOSTERONE REPLACEMENT THERAPY ON PHASIC CONTRACTIONS AND ACETYLCHOLINE RELEASE IN RAT BLADDER

Hypothesis / aims of study

In men testosterone levels decrease by 1% per year after the age of 40, with around 12% of men over this age showing androgen deficiency (1). Recent evidence suggests that low testosterone may play a role in bladder dysfunction. Androgen receptors are present on the urothelium, detrusor and autonomic nerves of the bladder (2) and clinically low androgen levels have been associated with urodynamic detrusor overactivity, that is alleviated by testosterone replacement therapy (TRT) (3). The urothelium has a critical role in bladder function, releasing a number of mediators including ATP, Ach and prostaglandins during bladder filling, which act on the underlying afferent nerves, detrusor muscle and interstitial cells. However, the precise effect of a decline in testosterone on these pathways is still unclear. The aim of the present study was to investigate the effect of low testosterone on mediator release (Ach and ATP) from isolated whole bladders and contractility of bladder strips from an orchiectomised rat model and determine the effect of testosterone replacement.

Study design, materials and methods

Male Wistar rats were castrated (orchiectomy) at 8 weeks of age by surgical bilateral orchiectomy under anaesthesia. 3 days later animals were treated with TRT (Sustanon 250 (Schering-Plough), mixed testosterone esters, 20µl, intramuscularly, every 21 days) for 8 weeks. Control rats were sham operated and received vehicle only. Isolated whole bladders were cannulated with a two-way cannula via the urethra and mounted in customised tissue baths containing gassed Krebs (95% O_2/5% CO_2) at 37°C. Bladders were distended by intravesical infusion of isotonic saline (150 µl/min) and subjected to low (275µl volume) and maximal distension (up to 2.85ml, 25mmHg). Intravesical contents were collected for measurement of Ach and ATP using commercially available kits (Amplex Red and Luciferin-Luciferase, Molecular Probes). Isolated bladder strips were mounted in tissue baths in Krebs solution. Amplitude and frequency of phasic contractions were recorded along with relaxation and contraction responses to isoprenaline, carbachol and electrical field stimulation (EFS) (1-40Hz, 0.01ms duration, 40V for 5s every 100s). All data are expressed as mean ± SEM. Data was compared via ANOVA with Bonferroni post hoc test; $P<0.05$ was considered significant.

Results

Orchiectomised animals showed significantly reduced serum testosterone compared to sham controls (2.69±0.22nM vs 18.48±2.14nM, n=7-8, $P<0.01$) and gained significantly less weight than sham controls over the 8 week period (final weight 443.14±17.82g vs 518.75±12.45g, $P<0.001$). Testosterone replacement to physiological levels (TRT) (11.11±1.10nM, n=8) prevented the reduced weight gain (518.25±16.83g). Intraluminal Ach release from isolated whole bladders following maximal distension was significantly less than release following low distension in all animal groups (Fig. 1). For both low and maximal distensions Ach release was significantly less in bladders from orchiectomised animals compared to those from sham controls ($P<0.05$, $P<0.01$ respectively) and this was prevented by TRT (Fig. 1). Intraluminal ATP release was similar in bladders from all animals. Amplitude and frequency of phasic activity was similar in isolated bladder strips from orchiectomised animals, although TRT significantly decreased amplitude of phasic activity compared to sham controls and orchiectomised animals (Fig. 2, $P<0.01$, $P<0.001$ respectively). The acetylcholinesterase inhibitor neostigmine significantly ($P<0.001$) increased the amplitude of phasic activity in bladder strips from orchiectomised animals, and this was only partially prevented by TRT (Fig. 2). Relaxation responses to the β-adrenoceptor agonist isoprenaline were similar in bladder strips from all animal groups, as were muscarinic receptor mediated contractile responses to carbachol and nerve-evoked contractions to EFS.

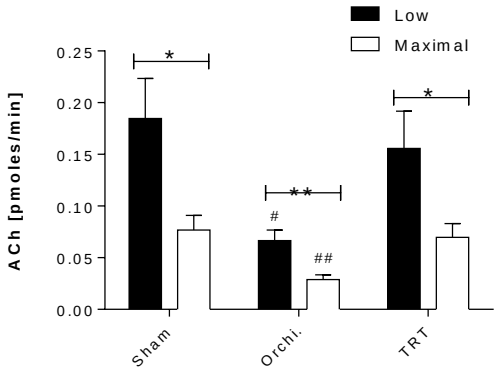

Fig.1. Intraluminal Ach release in response to low and maximal distension in isolated whole bladders from sham control, orchiectomised and TRT animals (n=7-8), $^{\#}P<0.05$, $^{\#\#}P<0.01$ vs sham, $^{*}P<0.05$, $^{**}P<0.01$ vs low distension.

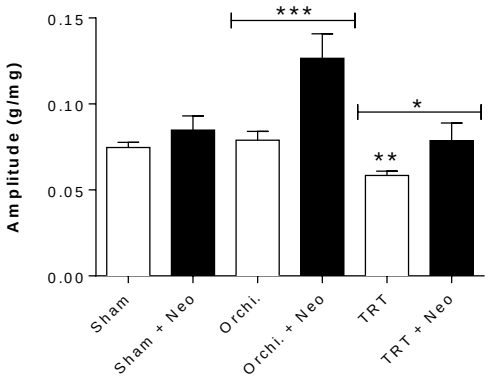

Fig. 2. Amplitude of phasic activity in the presence and absence of neostigmine in isolated bladder strips from sham control, orchiectomised and TRT animals (n=14-16), ** P<0.01 vs sham, *P<0.05, ***P<0.001 vs absence of neostigmine.

Interpretation of results

Intraluminal Ach release in response to maximal distension of bladders was less than that following low distension, suggesting possible intraluminal breakdown by acetylcholinesterase during the longer distension period. Orchiectomy of 8 weeks duration resulted in decreased Ach release, and this was accompanied by an increased effect of the acetylcholinesterase inhibitor neostigmine on phasic activity of isolated bladder strips, which suggests that there may be increased intraluminal breakdown of Ach within the bladder under conditions of low testosterone. Since agonist and nerve-evoked contractility of the detrusor were unaffected by 8 weeks of orchiectomy, this may suggest that alterations in urothelial signalling are an early effect of a decline in testosterone and may contribute to age-related bladder dysfunction.

Concluding message

Low testosterone due to orchiectomy causes decreased Ach release in the rat bladder, which may be related to an increased breakdown by acetylcholinesterase. These results indicate a role for testosterone in normal urothelial function and suggest that alterations in urothelial signalling may occur as a result of the age-related decline in testosterone and contribute to the pathophysiology of bladder dysfunction.

References

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Disclosures

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