

LOW TESTOSTERONE CAUSES ENHANCED RELEASE OF ATP FROM UROTHELIAL CELLS IN VITRO

Hypothesis / aims of study

Overactive bladder is increasingly prevalent with age, although the underlying mechanisms remain to be elucidated. In men testosterone levels decrease by 1% per year after the age of 40 (1), with around 12% of men over this age showing androgen deficiency (2). There is evidence to suggest that low testosterone plays a role in bladder dysfunction (3) and it is possible that age-related alterations in bladder function are a consequence of reduced androgen levels and related to the pathophysiology of overactive bladder. The urothelium has been shown to play a critical role in bladder function, releasing a number of mediators including ATP, Ach and prostaglandins during bladder filling and stretch. These mediators act to excite or inhibit the afferent nerves, detrusor muscle and other suburothelial cells, including interstitial cells or myofibroblasts, although the precise effect of a decline in testosterone on these pathways is still unclear.

The aim of the present study was to determine the effects of low testosterone and testosterone replacement on mediator release (ATP, Ach and PGE₂) from urothelial cells *in vitro*.

Study design, materials and methods

RT4 human urothelial cells were treated with testosterone (10 – 300 nM; within the physiological to supraphysiological range) for 24 hours or for 5 days at 37°C. Immediately following treatment samples were prepared for analysis of basal and stimulated mediator release by incubating cell cultures in normal or hypotonic (50% normal [NaCl], to mimic stretch) Krebs solution respectively for 15 minutes. The level of Ach, ATP and PGE₂ in these samples was measured using commercially available kits, Amplex Red (Molecular Probes), Luciferin-Luciferase (Molecular Probes), prostaglandin E₂ EIA (Cayman Chemical Company), and compared to release from cells under low testosterone conditions (~1.5 nM, similar to castrated male). Cell viability was measured using a resazurin cell proliferation assay. Data was compared via ANOVA with Bonferroni post hoc test and P<0.05 was considered significant

Results

Cell proliferation and Ach release were found to be similar in urothelial cells under low testosterone conditions and those treated with testosterone for 24 hours or 5 days. Whilst basal release of ATP from urothelial cells was unaffected by low testosterone conditions, ATP release in response to hypotonic stimulation, to mimic stretch, was significantly greater in cells under low testosterone conditions compared to those treated with 30 nM testosterone (physiological concentration) for 24 hours (6.8 fold, p < 0.05). This was not evident following the 5 day treatment. In addition, PGE₂ release was unchanged following 24 hour treatments, but was significantly increased under both basal and stimulated conditions following 5 days treatment with a supraphysiological concentration of testosterone (300 nM).

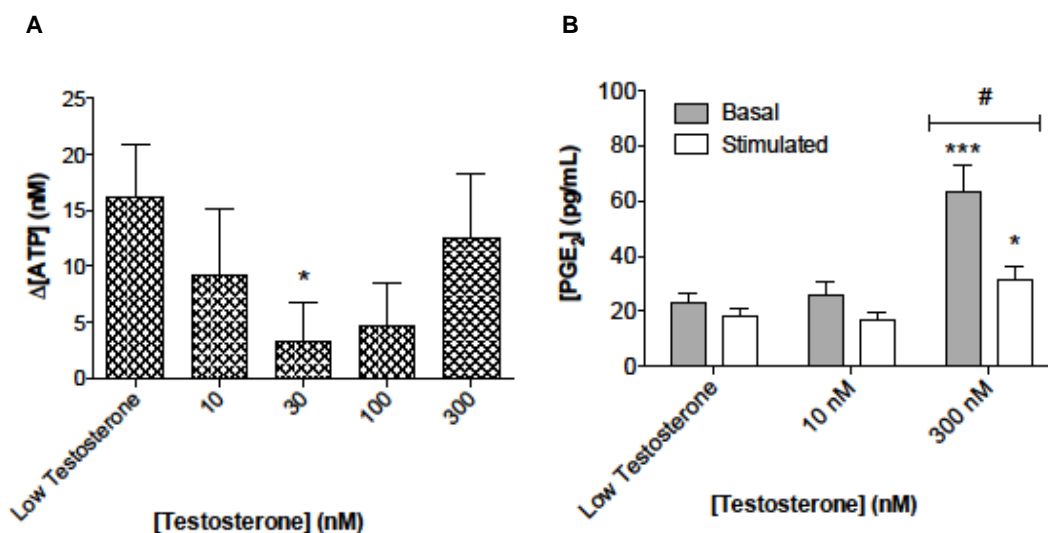


Fig.1. (A) Change in ATP release in response to hypotonic stimulation in 24-hour testosterone treated RT4 urothelial cells. Data is mean \pm SEM. (n=12), *P<0.05 vs. 30nM.

(B) Effect of 5-day testosterone treatment on basal and hypotonic stimulated PGE₂ concentrations in RT4 urothelial cells. Data is mean \pm SEM (n=6), ***P<0.001 vs. basal low testosterone; *P<0.05 vs. stimulated low testosterone; #P<0.05 vs. basal 300nM.

Interpretation of results

The findings indicate that release of ATP and PGE₂ from urothelial cells is sensitive to changes in testosterone concentration. Release of ATP from the urothelium in response to stretch activates bladder afferent nerves, which monitor distension, giving rise to bladder sensations such as fullness and urgency, and evoking urination. The enhanced release of ATP observed under low testosterone conditions suggests a possible role in the pathophysiology of overactive bladder. Alterations in urothelial

signalling may occur as a result of the age-related decline in testosterone and contribute to testosterone induced changes in bladder function.

Concluding message

Low testosterone causes enhanced release of ATP from human urothelial cells *in vitro*, whilst PGE₂ release is enhanced by supraphysiological levels of testosterone. 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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3. Koritsiadis et al., 2008 Androgens and bladder outlet obstruction: a correlation with pressure-flow variables in a preliminary study. BJU International, 101(12): 1542-1546.

Disclosures

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