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TADALAFIL ATTENUATES HYPOTONICITY-INDUCED CA2+ INFLUX VIA TRPV2 AND TRPV4 CHANNELS AS WELL AS ATP RELEASE IN PRIMARY BLADDER UROTHELIAL CELL CULTURES.

Hypothesis / aims of study
Phosphodiesterase 5 (PDE5) inhibitor, tadalafil, improves lower urinary tract symptoms suggestive of benign prostatic hyperplasia. The mechanism is believed to smooth muscle relaxation, increased blood perfusion and modulation of sensory stimuli via increased activity of the NO/cGMP/protein kinase G pathway. However study for tadalafil in urothelium is few, here we investigated the distribution of PDE5, as well as the molecular mechanism for tadalafil in signal transduction focusing on transient receptor potential (TRP) channels and ATP release of bladder urothelium.
Study design, materials and methods
All experiments were performed by using10-12 week old male Sprague-Dawley rats and C57BL/6 mice. PDE5 expression in rat bladder tissues and cultured primary rat bladder urothelial cells was evaluated using immunochemistry and western blot assays. Ca^{2+} influx in cells exposed to isotonic solution, hypotonic solution, a selective transient receptor potential vanilloid 2 (TRPV2) channel agonist (cannabidiol), a selective TRPV4 channel agonist (GSK1016790A), a TRP cation channel melastatin 7 (TRPM7) channel agonist (PIP2) or a purinergic receptor agonist (ATP) in the presence or absence of 10 μM tadalafil was evaluated using calcium imaging techniques. We also evaluated stretch-induced changes in ATP concentration in the mouse bladder in the presence or absence of 100 μM tadalafil.
Results
Immunochemistry and western blotting demonstrated abundant expression of PDE5 in rat bladder urothelium (Fig.1) as well as primary rat urothelial cell cultures. Ca^{2+} influx responded to hypotonic stimuli was significantly inhibited by pretreatment of tadalafil in primary rat bladder urothelial cell cultures. (Control (C): 13.1% v.s. tadalafil (T): 4.9%) (Fig.2). Ca^{2+} influx evoked by GSK (C: 74.7% v.s. T: 68.8%) or CAD (C: 52.2% v.s. T: 25.9%) was significantly inhibited by pretreatment of tadalafil. While Ca^{2+} influx caused by the stimulation of ATP (C: 49.6% v.s. T: 49.0%) could not be attenuated. PIP2 at the concentration of 4μM did not evoke Ca^{2+} influx. ATP release in the tadalafil pretreated-bladder was significantly smaller than control bladder.
Interpretation of results
Tadalafil attenuates Ca^{2+} influx via TRPV4 and TRPV2, and inhibits ATP release in the bladder urothelium.
Concluding message
Tadalafil functions as an inhibitor of urothelial signal transduction.

Figure 1

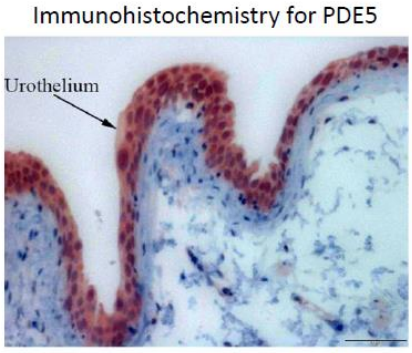
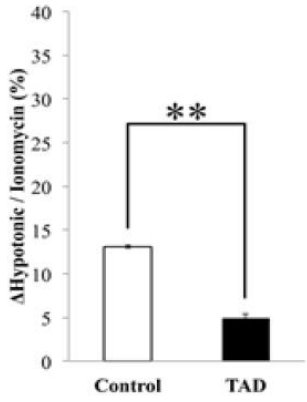


Figure 2



Ca^{2+} response to hypotonic osmolarity

Disclosures
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