Tadalafil attenuates hypotonicity-induced Ca^{2+} influx via TRPV2 and TRPV4 channels as well as ATP release in primary bladder urothelial cell cultures.

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Introduction & Objectives

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 (1). The bladder urothelium is presumed to be involved in sensory mechanisms activated in response to physical and chemical stimuli (2). Urothelial cells express several receptors/ion channels including transient receptor potential (TRP) channels and secrete ATP capable of modulating, activating or inhibiting sensory neurons (2). 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 TRP channels and ATP release of bladder urothelium.

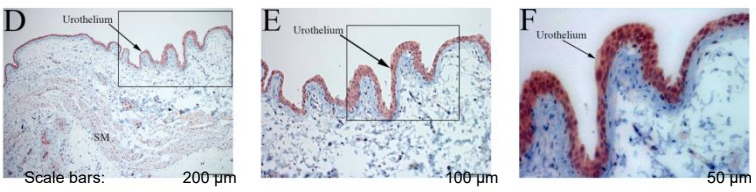
Methods

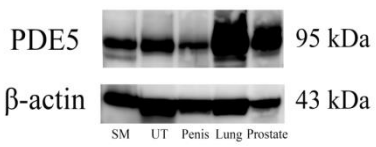
Animals 10-12 week-old male Sprague-Dawley rats and C57BL/6 mice were used in all animal experiments. All procedures were conducted in accordance with the policies of the Institutional Animal Care and Use Committee of the University of Yamanashi .

Immunohistochemistry: Rat tissue was fixed with 4% PFA, cryosectioned (3 μM) and mounted on glass slides. Sections were incubated with primary antibody (rabbit anti-PDE5A [1:50] [Santa Cruz Biotechnology]) and subsequently, incubated with goat anti-rabbit HRP (1:1,000) (Santa Cruz Biotechnology) . Samples were examined using an Olympus BX53 microscope.
Western blot analysis: Tissue specimens and primary rat urothelial cell cultures were homogenized using an M-PER mammalian protein extraction reagent. Images were captured using an Image Quant LAS4000 imager. Anti-PDE5A (1:500) (Santa Cruz Biotechnology) and anti-β actin (1:20,000) (Santa Cruz Biotechnology) .
Primary urothelial cell cultures : Whole bladders were harvested from anesthetized rats, and urothelial cells were prepared as previously described (3).
Intracellular Ca^{2+} concentration: Measuring using a Ca^{2+} imaging system as previously described (3). Ca^{2+} influx was evaluated under exposure to isotonic (340mOsm), hypotonic (200mOsm), cannabidiol (CAD, TRPV2-selective agonist) (10mM), GSK1016790A (GSK, TRPV4-selective agonist) (30nM), ATP (P2X and Y agonist) (5mM) and PIP2 (TRPM7 agonist) (4mM) stimuli respectively with pretreated tadalafil (10mM) or the vehicle control (acetonitrile).
***In vivo* ATP levels :**To quantify ATP release in vivo, 50 μL of saline supplemented with ARL 67165 (100 μM) was slowly injected into ureter-ligated bladders of control or tadalafil -treated mice via the urethra. The saline was subsequently collected, and ATP concentration was measured using a luciferase reagent (4).
Statistical analysis: All values are expressed as the mean ± SEM. The unpaired t-test was used for statistical analysis. For all analyses, P values of <0.05 were considered significant.

Results

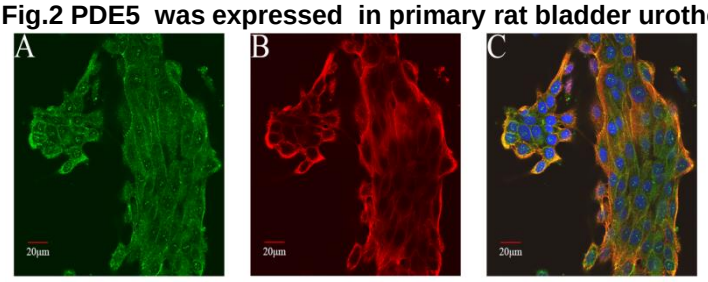
Fig.1 PDE5 was expressed in the rat bladder urothelium



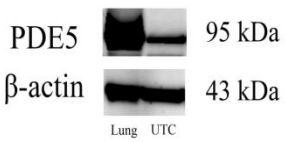


SM: smooth muscle
UT: urothelial tissue

Fig.2 PDE5 was expressed in primary rat bladder urothelial cell cultures



PDE5 Cytokeratin-7 Merge



UTC: urothelial cell cultures

Fig.3 Tadalafil-mediated inhibition of hypotonicity-induced Ca^{2+} influx

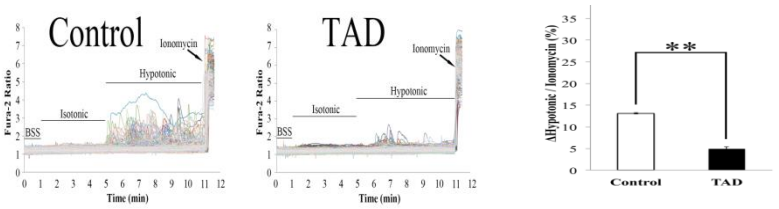


Fig.4 Tadalafil-mediated inhibition of Ca^{2+} influx induced by TRPV2 or TRPV4 agonism

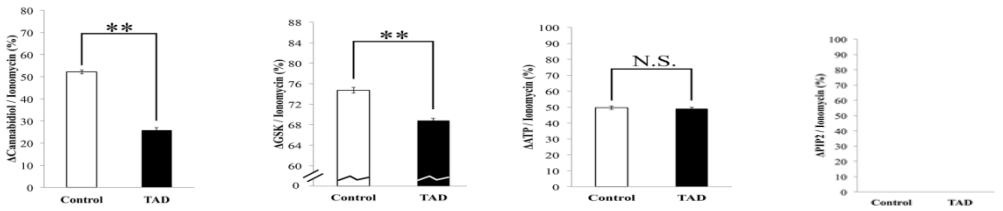
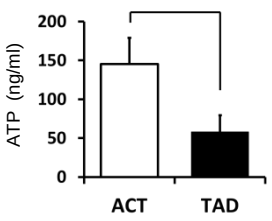


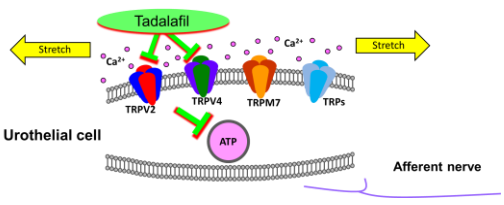
Fig.5 Tadalafil-mediated inhibition of stretch-induced ATP release in mouse bladder urothelium



Control mice (n = 9)
Tadalafil-treated mice (n = 8)

Conclusions

Tadalafil attenuated Ca^{2+} influx via TRPV4 and TRPV2 channels and inhibited ATP release in bladder urothelium. Tadalafil could act an inhibitory role in urothelial signal transduction. These novel insights into the mechanism underlying the effect of tadalafil on bladder sensation might inform the development of additional treatments for bladder disorders such as overactive bladder.



References

(1) Andersson KE, et al. Tadalafil for the treatment of lower urinary tract symptoms secondary to benign prostatic hyperplasia: pathophysiology and mechanism (s) of action. Neurourology and urodynamics 2011
(2) Birder L, et al. How does the urothelium affect bladder function in health and disease?: ICI-RS 2011. Neurourology and urodynamics 2012
(3) Mochizuki T, et al. The TRPV4 cation channel mediates stretch-evoked Ca2+ influx and ATP release in primary urothelial cell cultures. Journal of Biological Chemistry 2009
(4) Nakagomi H,. et al . Urothelial ATP exocytosis: regulation of bladder compliance in the urine storage phase. Sci Rep 2016

Conflict of Interest Disclosure
I have no potential conflict of interest to report