

Sildenafil relaxes human detrusor by cGMP-independent signaling pathways

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OBJECTIVES

Lower urinary tract symptoms (LUTS) comprise voiding urinary symptoms which are related to urethral obstruction (obstructive component) and storage urinary symptoms which are more particularly related to bladder dysfunction (irritative component) ¹.

Controlled clinical trials have recently demonstrated that daily phosphodiesterase 5 (PDE5) inhibitors including sildenafil indicated for the on demand treatment of erectile dysfunction (ED) are effective treatment for both obstructive and irritative LUTS suggestive of benign prostatic hyperplasia (BPH) in male patients with or without ED ²⁻⁴. However, the mechanism of action for these effects, more particularly on irritative symptoms, remains yet to be elucidated.

It is very tempting to speculate that PDE5 inhibitors could relieve bladder irritative symptoms through direct action on detrusor smooth muscle. A recent controlled pilot study in spinal cord injured male patients with neurogenic detrusor overactivity which has reported a significant improvement of urodynamic parameters with vardenafil administration ⁵, supports an effect on bladder function.

References

1. Chapple CR, Roehrborn CG. *Eur Urol* 2006;49:651-659; 2. McVary KT, et al *J Urol* 2007;177:1401-1407; 3. McVary KT, et al *J Urol* 2007;177:1071-1077; 4. Stief CG, et al *J Urol* 177[4 suppl]. 2007; 5. Gacci M, et al *J Urol* 2007;178:2040-2043.

The aim of this study was to assess the ability of sildenafil to relax human bladder tissue and to investigate the signaling pathways that could be involved in this sildenafil-mediated relaxation.

MATERIALS & METHODS

Human detrusor strip preparation

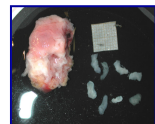
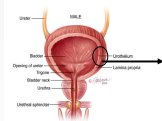
Bladders samples were obtained from 10 donors undergoing cystectomy for bladder cancer with no known bladder dysfunction according to their medical chart. Serosal and mucosal layers were removed from the bladder sample, and detrusor strips were mounted in 5 ml organ baths filled with Krebs-HEPES buffer maintained at 37°C and continuously bubbled with 95%O₂-5%CO₂. The strips were connected to force transducers for isometric tension recordings (Piodyn Controls Ltd, UK). Following amplification, the tension changes were computerized via MacLab™/8 using Chart™ 5 software (AD Instruments Ltd).



In vitro contractile experiments

After an equilibration period, concentration-response curves for sildenafil (from 10⁻⁹M to 3.10⁻⁶M) were generated in the presence or absence of selective blockers of the cGMP/ cAMP pathways or K⁺ channels on carbachol (10⁻⁶M)-induced precontraction.

ODQ (10 μM) : guanylate cyclase inhibitor
MDL-12,330A (10 μM) : adenylate cyclase inhibitor
Glibenclamide (10 μM) : K_{ATP} channel blocker
Iberritoxin (30 nM) : BK_{Ca} channel blocker
Apamin (100 nM) : SK_{Ca} channel blocker



Data Analysis

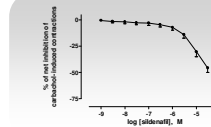
Data were expressed as mean ± SEM for N experiments corresponding to N = bladder samples obtained, from N different patients. Statistical comparisons of the CRC were performed with a two-way ANOVA statistic analysis test and Bonferroni's post-test. In case of interaction between the two factors (concentration and drug) with the two-way ANOVA statistic analysis, a modified Student's t-test with the Bonferroni adjustment for multiple comparisons was performed. P values < 0.05 were considered statistically significant. Statistical analysis was performed with GraphPad Prism® 4.03 software.

CONCLUSIONS

- ✚ The relaxing effect exerted by sildenafil on carbachol-precontracted human bladder tissue does not rely on a cGMP-signaling pathway and is thus probably not mediated through the inhibition of PDE5 isoenzymes.
- ✚ Conversely, this relaxant effect is dependent on a cAMP-signaling pathway. In addition, K_{ATP}, BK_{Ca} and SK_{Ca} channels are also involved in this relaxing effect.
- ✚ The exact mechanism by which sildenafil relaxes human detrusor deserves further investigation

RESULTS

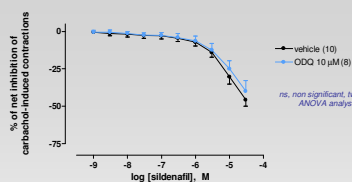
Effect of sildenafil on carbachol-precontracted human detrusor strips



➔ Sildenafil induced a significant concentration-dependent relaxation of carbachol-induced contraction of human bladder strips when compared to vehicle. The maximal effect of sildenafil was -45.7±4.2 % of inhibition of carbachol-induced contractions.

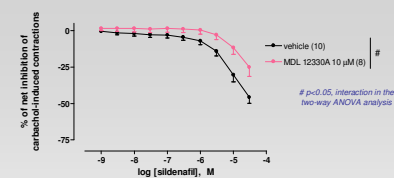
By which mechanism of action does sildenafil relax human bladder tissue ?

cGMP-dependent mechanism of action?

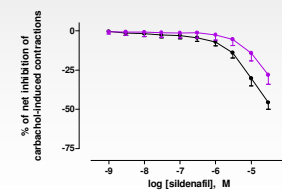


➔ Sildenafil inhibits carbachol-induced human bladder contractions via a cGMP-independent mechanism pathway

cAMP-dependent mechanism of action?



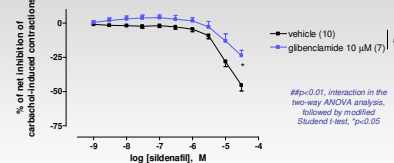
➔ Sildenafil seems to partly inhibit carbachol-induced human bladder contractions via a cAMP-dependent mechanism pathway



➔ Sildenafil also exerts its inhibitory effect by another pathway, independent of cGMP or cAMP signaling pathways, since some relaxing activity remains in presence of both MDL 123,330A and ODQ

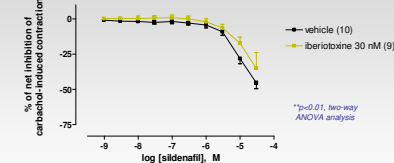
K⁺ channel-dependent mechanism of action ?

*K_{ATP} channels-dependent mechanism of action?



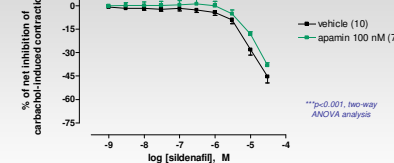
➔ K_{ATP} channels are involved in the relaxation elicited by sildenafil on human detrusor tissue

*BK_{Ca} channels-dependent mechanism of action?



➔ BK_{Ca} channels are involved in the relaxation elicited by sildenafil on human detrusor tissue

*SK_{Ca} channels-dependent mechanism of action?



➔ SK_{Ca} channels seem to be slightly involved in the relaxation elicited by sildenafil on human detrusor tissue