

## THE EFFECTS OF SILDENAFIL ON BLADDER CONTRACTILITY AND NEURONAL ATP RELEASE IN THE MOUSE URINARY BLADDER

### Hypothesis / aims of study

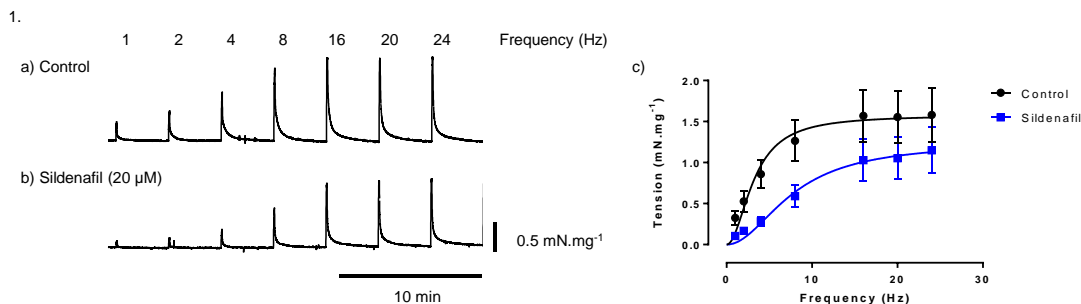
Current treatments for lower urinary tract symptoms (LUTS) associated with bladder diseases have many setbacks, including limited efficacy and adverse side effects. Phosphodiesterase type 5 (PDE5) inhibitors like sildenafil (Viagra®), which are clinically used to treat erectile dysfunction, have been found to also alleviate LUTS. The ability of PDE5 inhibitors to directly relax smooth muscle has been described [1], however, a complete understanding of its action on the bladder remains unclear. We are investigating the effects of sildenafil on several potential mechanisms involved in peripheral control of bladder function. In this study, the effects of sildenafil on nerve-mediated contractions of the isolated mouse bladder, and neuronal neurotransmitter release, were characterised. A comprehensive description of how sildenafil reduces detrusor contractile function should give insight into pathology of bladder diseases and suggest a potential therapeutic role of PDE5 inhibitors in their treatment.

### Study design, materials and methods

Bladders were removed from euthanised young (12 weeks) and aged (24 months) mice and detrusor strips tied to an isometric force transducer in a horizontal superfusion chamber. Contractures were generated by carbachol (CCh, 1  $\mu$ M) added to the superfusate. Nerve-mediated contractions (tetrodotoxin-sensitive) were generated by electrical field stimulation (EFS: 0.1 ms pulses, 1-24 Hz, 3-s train every 90-s). Contraction amplitude was normalised for tissue preparation weight ( $\text{mN}\cdot\text{mg}^{-1}$ ). Force-frequency relationships were used to determine maximum tension ( $T_{\text{max}}$ ), the frequency to attain  $T_{\text{max}}/2$  ( $f_{1/2}$ , Hz), and the ratio at low and near maximum frequencies ( $T_{2/20}$ ). The quantity of adenosine triphosphate (ATP) released (pmoles) into the superfusate during EFS was measured directly using a firefly luciferin-luciferase assay. A protocol of pelvic nerve stimulation using an arterially-perfused mouse model was used [2] to investigate nerve-mediated bladder pressure changes *in situ*. Data are means  $\pm$  SD, n=number of experiments. Differences between data sets were subjected to ANOVA with *post hoc* Student's t-tests; the null hypothesis was rejected at  $p < 0.05$ .

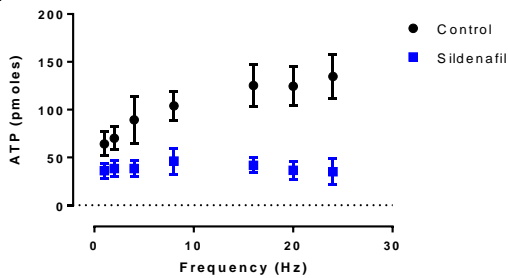
### Results

Sildenafil (1 – 30  $\mu$ M) significantly reduced peak CCh-induced contractions, from  $0.47 \pm 0.05$  to  $0.18 \pm 0.03$   $\text{mN}\cdot\text{mg}^{-1}$  at 30  $\mu$ M sildenafil ( $p < 0.001$ ,  $n=8$ ), with an  $\text{IC}_{50}$  value of  $17.5 \pm 2.0$   $\mu$ M. Sildenafil (20  $\mu$ M) also reduced EFS-stimulated contractions, but also significantly increased the  $f_{1/2}$  to the right, from  $3.31 \pm 0.47$  to  $7.56 \pm 0.92$  Hz in young mice ( $p < 0.01$ ,  $n=8$ ), and from  $1.92 \pm 0.67$  to  $7.65 \pm 0.42$  Hz in aged mice ( $p < 0.01$ ,  $n=5$ ) – Figure 1.  $T_{2/20}$  values were also significantly decreased upon the addition of sildenafil, from  $0.33 \pm 0.06$  to  $0.10 \pm 0.02$  in young mice ( $p < 0.01$ ,  $n=8$ ), and from  $0.61 \pm 0.14$  to  $0.08 \pm 0.01$  in aged mice ( $p < 0.05$ ,  $n=5$ ). These results show that sildenafil reduced EFS-stimulated contractions more at low frequencies, compared to high frequencies.  $\alpha, \beta$ -me-ATP (10  $\mu$ M), an ATP analogue, was used to desensitise purinergic receptors and leave only acetylcholine-mediated EFS contractions – sildenafil (20  $\mu$ M) had no significant effects on  $T_{\text{max}}$ ,  $f_{1/2}$ , or  $T_{2/20}$  ( $p > 0.05$ ,  $n=6$ ). By contrast, in the presence of atropine (1  $\mu$ M), to inhibit acetylcholine (ACh)-mediated EFS contractions, sildenafil (20  $\mu$ M) significantly reduced  $T_{\text{max}}$  from  $0.79 \pm 0.11$  to  $0.64 \pm 0.12$   $\text{mN}\cdot\text{mg}^{-1}$ , in comparison to the effects of atropine alone ( $p < 0.05$ ,  $n=6$ ). Sildenafil also significantly reduced ATP release during EFS-stimulated contractions in young mice ( $n=8$ ); from  $70.1 \pm 12.3$  pmoles to  $38.5 \pm 8.5$  pmoles at 2 Hz stimulation ( $p < 0.001$ ), and from  $124.6 \pm 20.2$  pmoles to  $36.6 \pm 9.2$  pmoles at 20 Hz stimulation ( $p < 0.01$ ) – Figure 2. Pelvic nerve stimulation in the arterially-perfused mouse model generated a frequency-dependent increase in *in situ* bladder pressure recordings at 1-24 Hz stimulation, and preliminary results demonstrate relaxation of nerve-mediated whole bladder pressure movements by sildenafil *in situ*, which is comparable to the effects *in vitro*.



**Figure 1.** The effect of sildenafil on EFS-stimulated contractions in intact bladder preparations young mice ( $n=8$ ). Traces are illustrated for (a) control and (b) 20  $\mu$ M sildenafil. (c) Force-frequency relationships in the absence and presence of sildenafil.

2.



**Figure 2.** The effect of sildenafil on neuronal ATP release in isolated bladder strips from young mice.

#### Interpretation of results

Sildenafil reduced the magnitude of nerve-mediated contractions in detrusor from young and aged mice. Although it reduced agonist-induced contractions, the data on nerve-mediated contractions suggests an additional effect on neurotransmitter release or its down-stream effects. Nerve-mediated contractions are dominated by ATP release at low frequencies, and ACh release at higher frequencies [3]. The fact that sildenafil had no action on ACh-dependent contractions (in the presence of  $\alpha, \beta$ -me-ATP) suggests that the local ACh concentration is less than  $10 \mu\text{M}$ , as it did suppress the CCh contracture. The predominant effect of sildenafil at low frequencies, the blockade of atropine-resistant contractions and its almost complete abolition of nerve-mediated ATP release all suggest that the agent has a specific effect on ATP neurotransmitter release or at least its persistence in the nerve-muscle junction. The particular pathways whereby this action is mediated are yet to be determined.

#### Concluding message

Sildenafil has a significantly greater effect on the low-frequency, purinergic-mediated contractions, and significantly suppressed neuronal ATP release. These results demonstrate a novel action of sildenafil to selectively inhibit ATP release from nerve-terminals innervating detrusor smooth muscle, a neurotransmitter associated with overactive bladder conditions in humans.

#### References

1. Oger S, Behr-Roussel D, Gorny D, Lebret T, Validire P, Cathelineau X, Alexandre L, Giuliano F. Signalling pathways involved in sildenafil-induced relaxation of human bladder dome smooth muscle. *Br J Pharmacol.* 2010;160(5):1135-43.
2. Ito H, Pickering AE, Igawa Y, Kanai AJ, Fry CH, Drake MJ. Muro-Neuro-Urodynamics; a Review of the Functional Assessment of Mouse Lower Urinary Tract Function. *Front Physiol.* 2017;8:49.
3. Pakzad M, Ikeda Y, McCarthy C, Kitney DG, Jabr RI, Fry CH. Contractile effects and receptor analysis of adenosine-receptors in human detrusor muscle from stable and neuropathic bladders. *Naunyn Schmiedebergs Arch Pharmacol.* 2016;389(8):921-9.

#### Disclosures

**Funding:** NIH R01 DK098361 **Clinical Trial:** No **Subjects:** ANIMAL **Species:** Mouse **Ethics Committee:** University of Bristol Ethics Committee