

EFFECTS OF SILDENAFIL ON BLADDER AND URETHRAL FUNCTION IN A NOVEL VOIDING MOUSE MODEL: THE DECEREBRATE ARTERIALLY-PERFUSED MOUSE (DAPM) PREPARATION

Hypothesis / aims of study

Phosphodiesterases type 5 inhibitors (PDE5-Is) increase levels of cyclic guanosine monophosphate (cGMP) and can amplify nitric oxide (NO) signaling to cause smooth muscle relaxation peripherally. They are increasingly used to treat lower urinary tract symptoms (LUTS). The proposed mechanisms improving LUTS include increased peripheral oxygenation, the relaxation of the bladder and prostate, and decreased afferent activity [1]. However, other potential, central mechanisms remain to be investigated. Previously, we have reported a decerebrate, arterially perfused rat (DAPR) preparation which allows consistent filling and voiding responses [2]. The present study aimed to develop a decerebrate arterially perfused mouse (DAPM) preparation and investigate the effects of PDE5-Is on bladder and urethral function of mice.

Study design, materials and methods

CD1 male mice (2 -3 months, 34-36 g), were terminally anaesthetized with isoflurane. The mice were rapidly cooled and then decerebrated at the pre-collicular level. The preparation was arterially perfused through the left ventricle with carbogenated, Ringer's solution at 31°C. A glass suction electrode was used to record from the left phrenic nerve, this activity gave a continuous index of preparation viability. To record external urethral sphincter (EUS) - electromyography (EMG), a glass suction electrode was placed on the proximal sphincter under direct visual control. A 27G needle was inserted into the bladder dome for pressure monitoring and infusion of 0.9 % saline at the rate of 25µl/min (Figure 1A).

Once the preparation was cardiovascularly 'tuned', a robust eupnoeic pattern of phrenic activity was stably observed for periods up to 3 hours. Functional micturition cycles were observed in response to infusion of 0.9% saline (Figure 1B). During the void, the bladder showed strong contractions and bursting activity of the EUS was observed.

To investigate the effect of sildenafil, (a PDE5-I), on the bladder and urethral function of mice, after obtaining baseline recordings of >5 voiding cycles, sildenafil was applied to the perfusate on an incrementing dose schedule every 4 voiding cycles (10 pM and 30 pM).

To investigate the peripheral effects of sildenafil directly on the bladder (excluding any drug effects on CNS), we established the DAPM and then removed the brain stem and pithed the spinal cord with a blunt wire. The micturition cycle was lost and the bladder became incontinent therefore to allow measures of bladder compliance when the urethra was clamped and saline was instilled into the bladder (25µl/min, to a maximal intra-vesical pressure of 15 mmHg). After control recording, sildenafil was applied into the perfusate and the recordings were repeated.

Results

In the DAPM, systemic application of sildenafil decreased the threshold pressure (at both 10 and 30pM, $P < 0.01$, $n = 6$) and increased the bladder compliance (30µM, 32%, $P < 0.05$) compared with vehicle (Figure 2). Sildenafil significantly increased the number of spikes during bursting activity of the EUS (Figure 2). In the pithed DAPM (without the control of CNS), Sildenafil significantly increase the bladder capacity at the dose of 30 pM ($P = 0.038$, compared with baseline ($n = 7$), Figure 3)

Figure 1

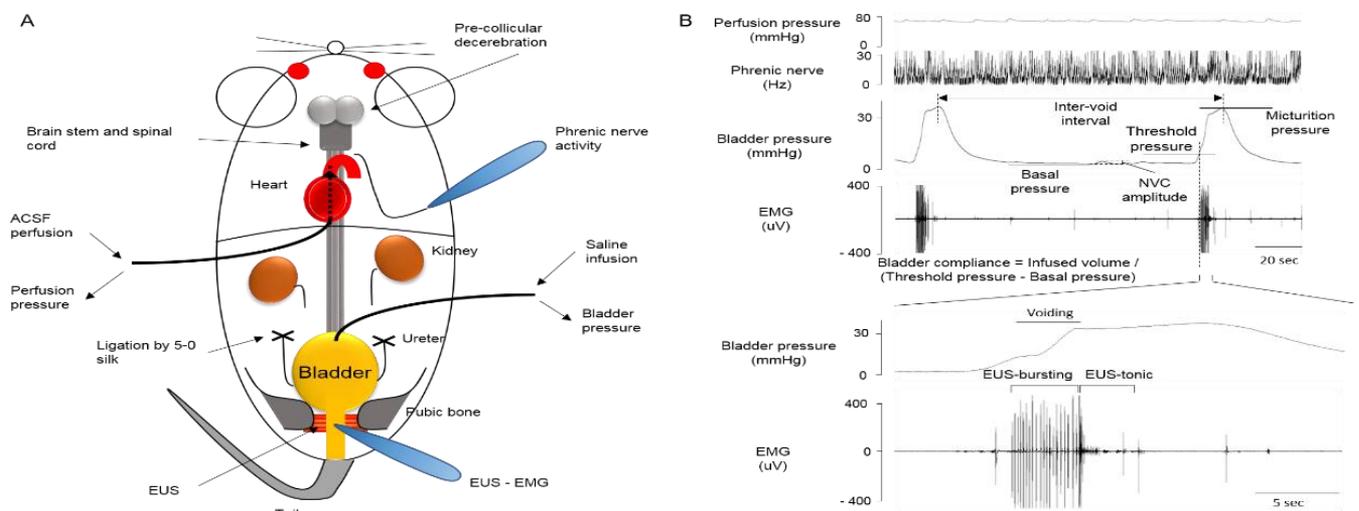


Figure 2

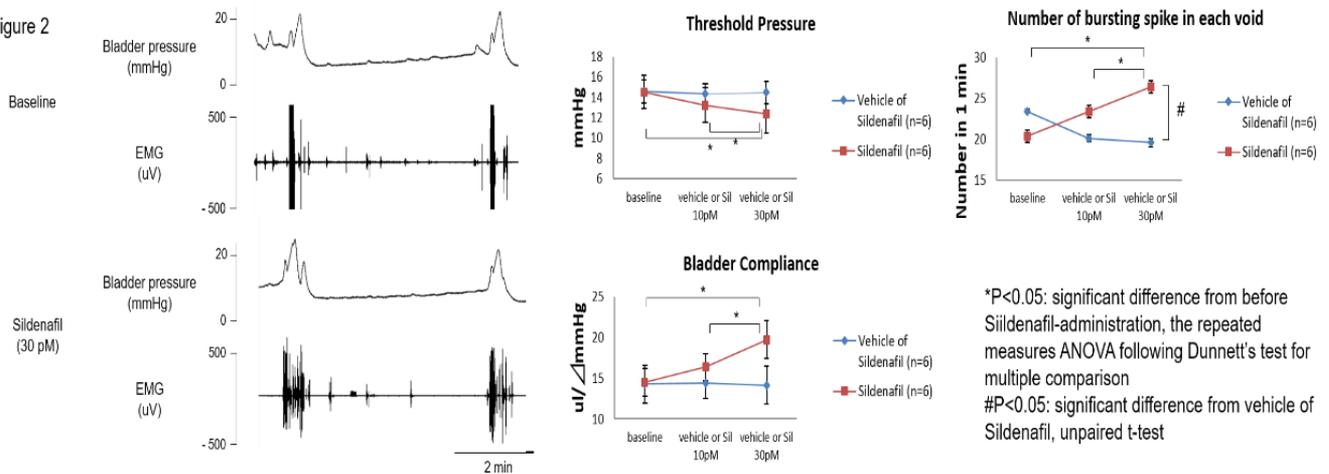
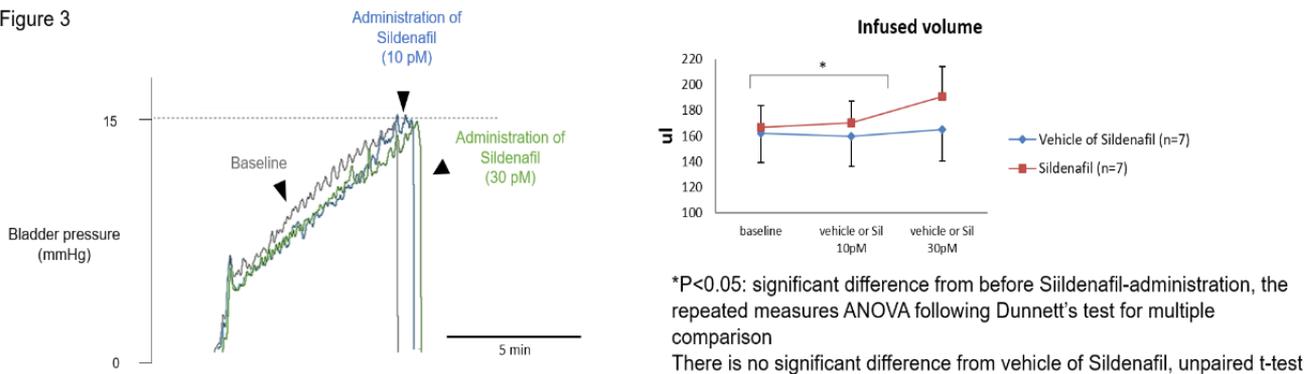


Figure 3



Interpretation of results

No study has previously assessed the effects of PDE5-Is on the activity of the EUS in voiding. This is first report to show that sildenafil increased EUS-EMG activity during voiding in mice. A previous immunofluorescence assessment identified that PDE5 was abundantly expressed in the striated muscle of the urethra and the levator ani muscle of rats, and this indicated that striated muscles are possibly regulated by PDE5. Sildenafil has a relaxing effect on the urethral muscle of mice and rats in organ bath studies. However, previous clinical evidence showed that PDE5-Is had few effects of increased urine flow rate, which indicated that this relaxant effect does probably not explain why sildenafil is useful in LUTS - our new finding may help to account for the beneficial effects on EUS activity and possibly central nervous system, especially sympathetic neural activity, which might improve LUTS symptoms.

In terms of bladder parameters, sildenafil decreased the threshold pressure and increased bladder compliance. The possible mechanism is decreased afferent firing of pelvic nerve. Some papers indicated that PDE5-Is and NO reduces mechanosensitive afferent activities of both A δ - and C-fibres elicited by bladder distension in the rat although investigation of afferent activity in mice is yet to be undertaken. The other possible mechanism is direct relaxation of the bladder which we were able to demonstrate in the pithed DAPM. This finding is consistent with previous reports and sildenafil has been reported to have relaxant effect on the human bladder and mouse detrusor showed relaxant response to soluble guanylyl cyclase stimulators [3].

Concluding message

The present study demonstrates the utility of a novel voiding mice preparation, DAPM, which allows the complete micturition cycle of mice to be investigated without the confound of anaesthesia. Using the DAPM we have shown a novel effect of sildenafil on the EUS which may be of relevance to its use in the treatment of LUTS.

References

- Gacci M, Andersson KE, Chapple C, Maggi M, Mirone V, Oelke M, et al. Latest Evidence on the Use of Phosphodiesterase Type 5 Inhibitors for the Treatment of Lower Urinary Tract Symptoms Secondary to Benign Prostatic Hyperplasia. *European urology*. 2016;70(1):124-33.
- Sadananda P, Drake MJ, Paton JF, Pickering AE. An exploration of the control of micturition using a novel in situ arterially perfused rat preparation. *Front Neurosci*. 2011;5:62.
- Bau FR, Monica FZ, Priviero FB, Baldissera L, Jr., de Nucci G, Antunes E. Evaluation of the relaxant effect of the nitric oxide-independent soluble guanylyl cyclase stimulator BAY 41-2272 in isolated detrusor smooth muscle. *European journal of pharmacology*. 2010;637(1-3):171-7.

Disclosures

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