FINAL PROGRESS REPORT

Grantor organization: International Continence Society

Grant type: International, multidisciplinary pilot research project (seed funding)

Project title: *Effects of PDE-5 Inhibition on Afferent Nerves and Interstitial Cells in Overactive Mouse Bladders*

Goals: A two centre international collaboration, between the US and UK, to study the therapeutic benefits and sites-of-action of the PDE-5 inhibitor, Sildenafil, on lower urinary tract pathophysiology. As a seed grant, an overriding goal was to obtain sufficient preliminary data to write for a joint National Institutes of Health R01 Grant.

Specific Objectives:

1st objective: Measure the effects of PDE-5 inhibition on afferent nerve firing *versus* neuropeptide release in bladder sheets with attached pelvic nerves (L6-S1) isolated from normal and spinal cord transected (T8-T9) mice.

2nd objective: Measure the effects of PDE-5 inhibition on spontaneous pacing/firing of interstitial cells in bladder wall cross-sections and cultured cells.

3rd objective: Measure the alterations in storage and voiding reflexes in normal mice before and after brainstem/spinal cord transection.

The principal investigators would like to thank the ICS for their support and point out that results obtained with the help of this seed funding permitted us to submit a joint application to the NIH. This R01 grant proposal (1 R01 DK098361-01) received very good scores for a first submission (impact: 30 and percentile: 28) and we are confident that our resubmission will be successful. Moreover, a joint publication based upon these studies is nearing submission.

Phosphodiesterase type-5 (PDE-5) inhibitors used clinically to treat erectile dysfunction also help lower urinary tract (LUT) symptoms, *e.g.*, in patients with benign prostatic hyperplasia. However, their therapeutic sites-of-action in LUT symptoms are unclear and their elucidationwere the objectives of this joint 18 month research effort.

Our first hypothesis was that PDE-5 inhibition decreases afferent nerve firing thereby ameliorating both neurogenic and myogenic overactivity. **Figure 1** shows the effects of sildenafil on stretch-induced afferent firing in normal and spinal cord transected (SCT) mouse bladder sheets from the Pittsburgh group. Bladders were stretched with a computer controlled stepper motor, and afferent firing was recorded from the associated S1 spinal roots. In contrast to control animals, SCT mouse bladders displayed large amplitude intrinsic contractions that stimulated afferent firing. Stretch-evoked afferent firing rates were also increased in comparison to control bladders. Sildenafil (1 μM) reduced afferent firing in response to intrinsic contractions and bladder stretch in SCT but not control mouse bladders. Contractile activity and baseline tension were also not altered by PDE-5 inhibition. Importantly, these data suggest that sensitized C-fibers, but not Aδ-fibers, are inhibited by sildenafil.

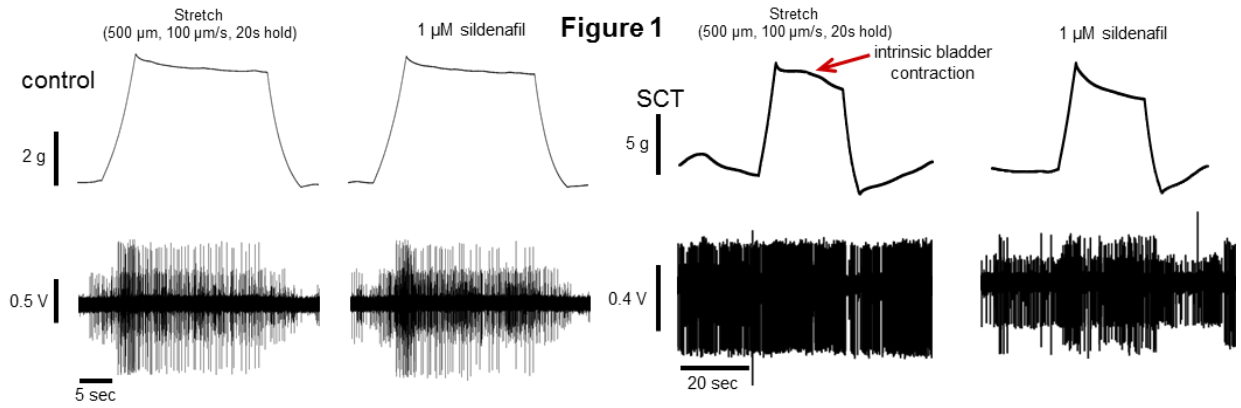


Figure 1

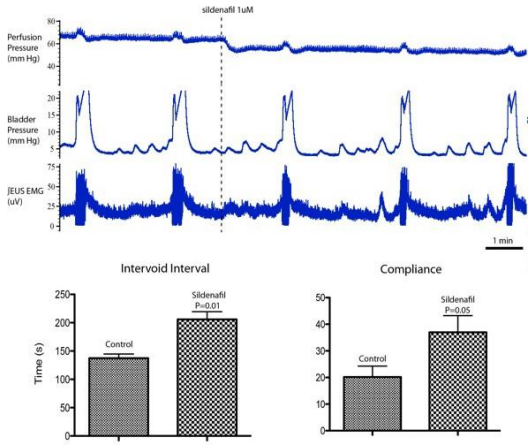
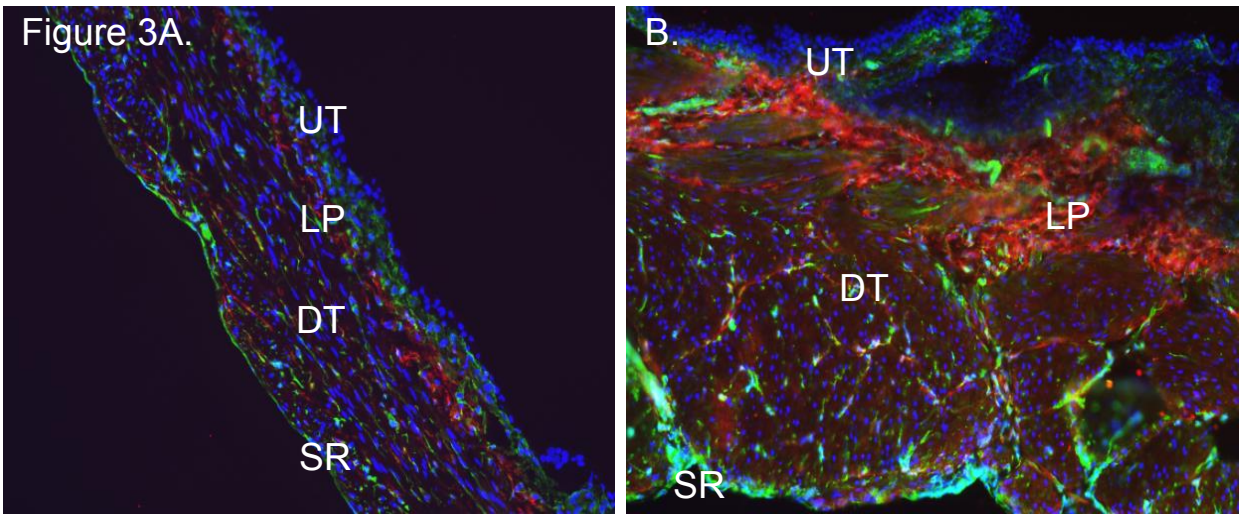


Figure 2 shows the influence of sildenafil in Bristol's decerebrate arterially perfused preparation. It importantly illustrates the interval between voids and filling-phase compliance (relationship between bladder pressure and volume) in a juvenile rodent, before and after 1 µM systemic sildenafil (at the dotted line). Both parameters increased following drug exposure, signifying improved storage function (*i.e.*, voiding pressures were unchanged, and there were no post void residuals). Commensurate with the enhanced striate function, was a reduction in overall afferent nerve activity during filling (not illustrated). At the time sildenafil was introduced systemically, there was a small drop in arterial perfusion (top line).

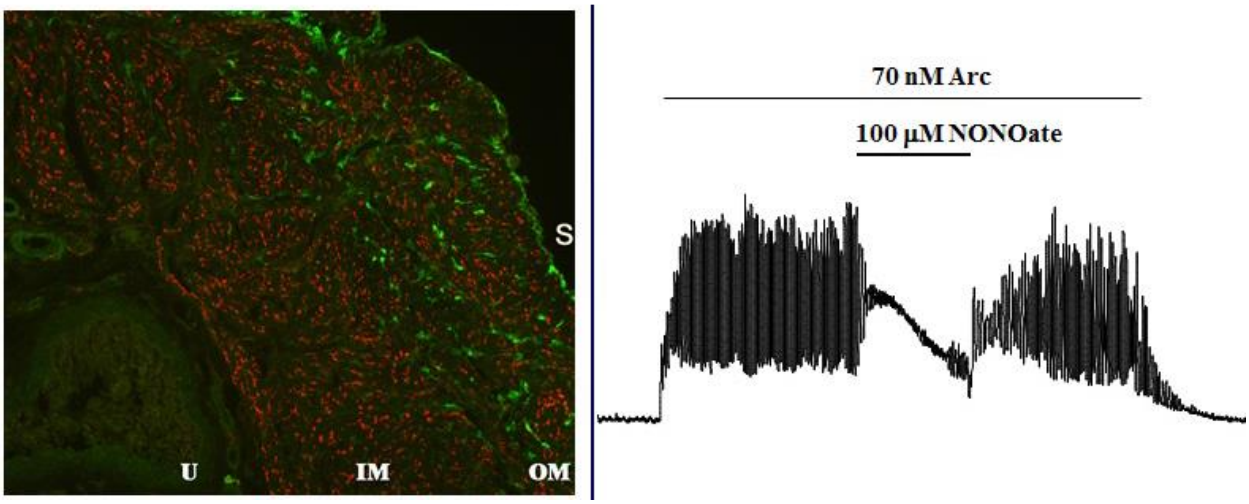
Our second hypothesis was that nitric oxide produced by the urothelium uncouples lamina propria interstitial cells decreasing their pacemaker activity and therefore spontaneous bladder contractions. This was linked to our third hypothesis that spinal cord transection results in interstitial cell hyperplasia such that existing nitric oxide levels are inadequate to uncouple these cells leading to detrusor overactivity. **Figure 3** from the Pittsburgh



group shows examples of normal (3A) and spinal cord transected (3B) rodent bladder cross-sections labeled for the interstitial cell markers, vimentin (green) and CD34 (red), magnification x100. Following spinal cord transection, there was significant remodeling of the bladder wall. Specifically, there was hypertrophy of the detrusor (DT) and hyperplasia of the urothelium (UT)

and interstitial cells (especially CD34 positive cells in the lamina propria, LP). The increase in the number of interstitial cells was greater than that of urothelial cells, which we propose decreases the inhibitory effects of nitric oxide on interstitial cells, thereby increasing their pacemaker activity which can drive intrinsic contractions contributing to detrusor overactivity.

Figure 4 from preceding work by the Co-PI shows the effects of a nitric oxide donor on intrinsic mouse bladder wall contractions. This figure demonstrates that nitric oxide profoundly alters the contractile function of the isolated bladder at capacity (pressure trace, right) and that nitric oxide donor elicits cGMP generation only in interstitial cells in the outer muscle layer (OM) (histology figure, left; U=urothelium, IM=inner muscle, S=serosa).



Significance of the Collaboration

This two center international collaboration drew upon complementary expertise to address key issues regarding the role of PDE-5 inhibitors on afferent nerves and interstitial cells in overactive bladder pathophysiology due to spinal cord injury. The University of Pittsburgh group has developed new *in vitro* approaches for studying LUT symptoms in the periphery in isolated tissues and cells, while the University of Bristol team has developed new *in situ* approaches for studying CNS reflexes in decerebrate arterially perfused mice. The findings obtained during these studies and future collaborations, given the synergy of the research teams, are likely to increase our knowledge of the role of PDE5 inhibitors in treating LUT dysfunctions.