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PHARMACOLOGICAL MANIPULATION OF AUTONOMIC BLADDER CONTROL IN A NOVEL UNANAESTHETISED DECEREBRATE RAT PREPARATION

Hypothesis / aims of study

We have recently developed a novel, non-anaesthetised *in situ* preparation of the whole rat with intact lower urinary tract (LUT) function [1,2]. The preparation allows the quantification of both evoked and natural voiding as well as of non-micturition contractions (NMCs). The aim of this study was to assess the autonomic control of these LUT functions and to pharmacologically manipulate this regulation in the preparation.

Study design, materials and methods

Female Wistar rats (40-80g) were anesthetized with halothane. Access to the bladder was achieved via a laparotomy, and the stomach, spleen and intestine were tied off and removed. The animal was immediately immersed in cold artificial CSF (aCSF) and decerebrated, at which point the anaesthetic was withdrawn. The preparation was placed in a recording chamber and arterially-perfused with carbogen-gassed, aCSF (32ºC) via a double lumen cannula inserted into the ascending aorta. The heart resumed beating immediately once perfusion commenced. Rhythmic respiratory muscle contractions were seen within minutes, as perfusion pressure reached 70mmHg.

A glass suction electrode was used to record activity from the phrenic nerve, used as a physiological indicator of preparation viability. A cannula, connected via a 3-way tap to a pressure transducer and syringe pump, was inserted into the bladder dome for filling and pressure monitoring. A bipolar glass suction electrode was positioned laterally on the proximal external urinary sphincter (EUS) to record EUS-EMG.

To assess autonomic involvement, ganglion blocker, hexamethonium (330µM) was systemically administered via the perfusate. To assess the role of cholinergic neurotransmission, the muscarninic antagonists, pirenzepine (M1>M3, M2; 0.1µM) and methoctramine (M2>M1>M3; 0.1µM) were also added systemically. In addition, b-adrenoceptor involvement was also tested by perfusion with isoprenaline, in incremental steps (1nM to 1µM). Apart from LUT specific parameters, the effects of these drugs on perfusion pressure, heart rate and phrenic nerve activity was also monitored.

Results

All preparations showed filling and voiding responses characteristic of the rat (rise in intraluminal pressure, high frequency oscillation of intraluminal pressure, followed by an iso-volumetric contraction and sharp decline). The external urethral sphincter showed tonic increase in activity that mirrored bladder pressure during filling. At void, bladder pressure oscillations were synchronous with high frequency bursting activity of the EUS. The mean threshold evoked-voiding pressure was 22±0.5 mmHg (n=10). The preparation also showed natural spontaneous voiding responses as fluid entered via the ureters (23±1 mmHg; n=12). In these preparations, inter-void interval was 3.5±0.6 min. NMCs were present in 11 out of 13 preparations, with mean amplitude and frequency of 3.8±0.6 mmHg and 2.3±1.1/min, respectively and duration of 10.9±0.7 s (n=12).

Ganglionic blockade with hexamethonium decreased voiding pressure, although EUS bursting activity during voiding was unchanged (Fig 1; n=4). Subsequently, the baseline intravesical pressure (3.7±0.8 mmHg) increased by 116% and the NMCs amplitude increased by 86% (Figure 1iii). During this period of ganglion block the EUS EMG activity increased during each NMC to maintain continence. Voiding continued, but occurred at a lower voiding pressure and was incomplete.

The b-adrenoceptor agonist, isoprenaline, caused a progressive reduction in voiding threshold pressure (Fig 2). At the highest 1µM dose, the threshold pressure was reduced by 19% (n=3) and NMCs were significantly attenuated in amplitude ($P<0.005$) (Fig 2). In addition, perfusion pressure incrementally dropped by 4-6 mmHg with each increase in concentration (presumably reflecting adrenoceptor mediated muscle vasodilation).

Muscarinic antagonists, pirenzepine and methoctramine increased the spontaneous intervoid interval 2-fold (n=4) and 3-fold (n=3),respectively. Pirenzepine but not methoctramine tended to reduce the frequency of NMCs.

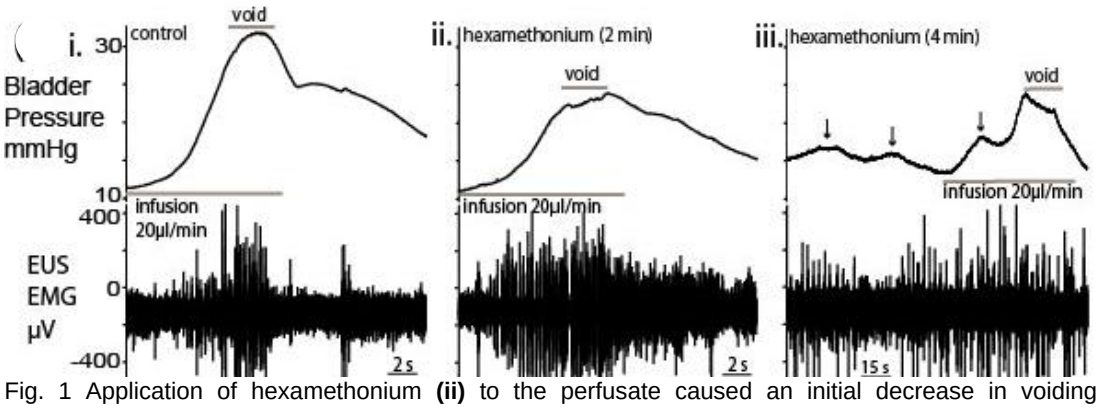

Fig. 1 Application of hexamethonium **(ii)** to the perfusate caused an initial decrease in voiding pressure, although the characteristic pressure trajectory and EUS bursting activity remained. **(iii)** Subsequently, NMCs (arrowed) became larger in amplitude, with associated EUS activity. Voiding was present, but with low voiding pressure.

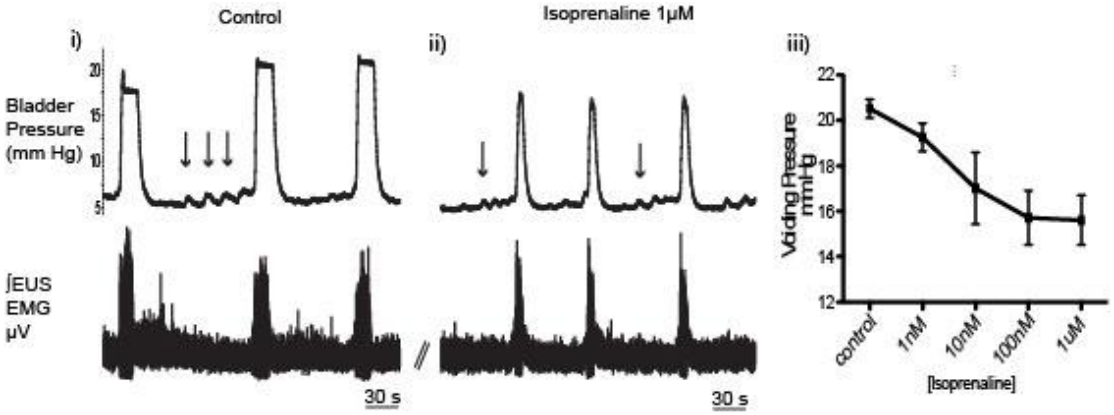

Fig 2. Natural filling and voiding responses in **i**) control and **ii)** presence of isoprenaline caused a decrease in NMC activity (arrowed) and voiding pressure. **iii**) Decrement of voiding pressure with each concentration of isoprenaline

Interpretation of results

These results confirm that the preparation allows the accurate quantification of both natural and evoked filling and voiding characteristics as well as NMC activity. The preparation has preserved autonomic tone, which regulates the normal bladder filling and voiding responses, as evidenced by the effect of ganglion blockade. In addition, the increased amplitude of NMCs suggests that they are tonically inhibited by autonomic control. The action of isoprenaline to also inhibit NMCs suggests that this may be a sympathetically mediated regulation. The influence of muscarinic receptors on lower urinary tact function is also confirmed in this preparation, where muscarinic blockade increased the inter-void interval (allowing increased bladder capacity). This may be consistent with a reduced excitability of the detrusor muscle.

Concluding message

This novel *in situ* whole rat preparation facilitates the pharmacological assessment of interventions targeted against measures of integrated lower urinary tract function, with the ease and control typically associated with reduced in vitro preparations. We anticipate that this will therefore prove to be a useful model for development and testing novel therapies.

References

1. Pickering AE and Paton JRF (2006) J Neurosci Methods 155(2): 260-71
2. Sadananda P, Drake MJ, Paton JRF, Pickering AE (2010) International Continence Society, Toronto

Specify source of funding or grant	**Astellas Pharma Inc.**
Is this a clinical trial?	**No**
What were the subjects in the study?	**ANIMAL**
Were guidelines for care and use of laboratory animals followed or ethical committee approval obtained?	**Yes**
Name of ethics committee	**University of Bristol Animal Ethics**