

CHARACTERISATION OF SPONTANEOUS CONTRACTILITY OF THE MUCOSAL LAYER OF GUINEA PIG BLADDER: COMPARISON WITH THE DETRUSOR AND INTACT BLADDER LAYERS

Hypothesis / aims of study

The urothelium is more than a passive barrier to urine and bacteria; it actively produces bioactive compounds in response to stimuli. Moreover, recent reports [1] show that the mucosal layer exhibits phasic spontaneous contractions; not unlike detrusor or whole bladder wall ('intact') preparations. The purpose of this study was to investigate the pharmacology of mucosal layer contractions, testing the hypothesis that spontaneous contractions (SpC) and slow, tonic contraction are of different origin.

Study design, materials and methods

The urinary bladder of male guinea pigs (400-500 g) was removed and its ventral wall was opened longitudinally from the bladder neck to the top of the dome. The mucosa and detrusor muscle layers were divided by microscope-guided careful dissection using iris scissors. Three types of tissue strip, confirmed by histology, were prepared; mucosal strips composed of urothelial and suburothelial layers; detrusor strips from which the mucosa was removed; intact strips. Each strip was 6-8 mm long and 1-2 mm wide. Preparations were mounted in a horizontal chamber, tied to an isometric force transducer and superfused with Tyrode's solution for 90 minutes. Baseline SpC was recorded for 10 minutes; followed by superfusion with agonists added to Tyrode's solution for a further 30 minutes when SpC and tonic contractions were recorded. The cross-sectional area (CSA) of strips was calculated as: $CSA = \text{weight (mg)} / \rho * \text{length (mm)}$, where ρ is the density of tissue ($1.05 \text{ g} * \text{cm}^3$) and the tension values were normalised by CSA. Following functional experiments, some tissue strips were fixed in 4% paraformaldehyde and embedded in paraffin blocks. Cross-sections were subsequently cut and stained with hematoxylin and eosin, and Masson's trichrome to estimate the percentage of muscle. Histological images were digitised and analysed using ImageJ. *Data analysis*; Area-under-the-curve (AUC) of SpC and tonic contractions were differentiated by using computer software, as indicated in Figure 1. Data are *medians* [25, 75% *interquartiles*]. Comparisons between groups were by Friedman's test with Dunn's *post-hoc* tests. The null hypothesis rejected at $p < 0.05$.

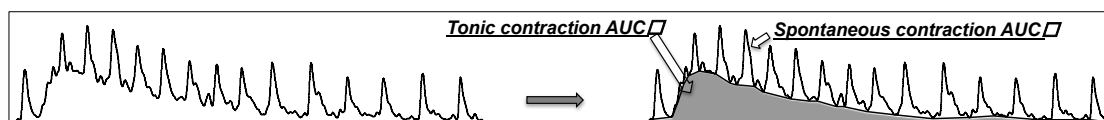


Figure 1 The algorithm to measure tonic and spontaneous contractions

Results

Baseline SpC; Typical baseline SpC traces are shown in Figure 2A. Mucosa strips contained much less muscle (4.5% [2.2, 6.2]) than detrusor (73.6 [72.7, 85.4]) or intact (63.2 [55.5, 71.4]) preparations (Figure 2B). AUC of baseline SpC was greatest in intact strips ($10.5 \text{ mN} * \text{mm}^{-2} * \text{min}$ [3.5, 21.9, $n=48$]) compared to detrusor (0.94 [0.47, 2.2, $n=45$]) and mucosa (4.5 [2.2, 7.6, $n=46$]). However, when AUCs of baseline SpC were normalised by % muscle area (Figure 1C), the mucosa preparations generated much greater SpC than detrusor layer.

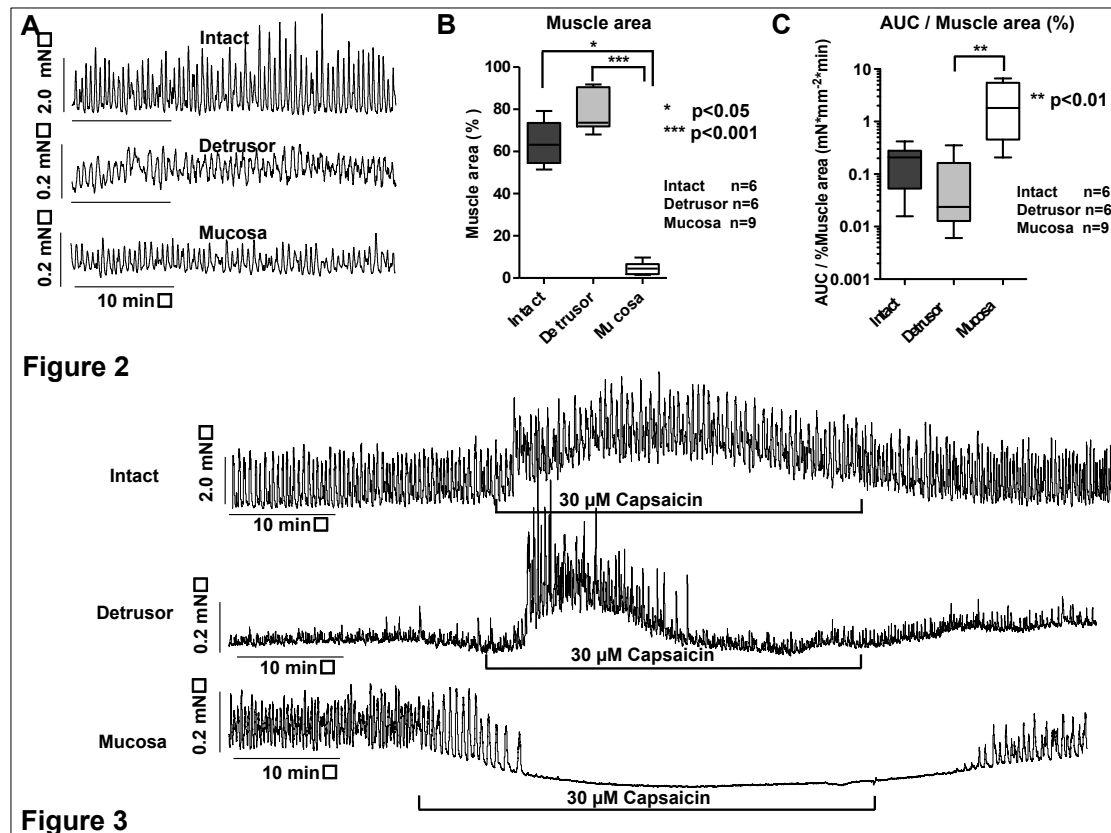
Effect of investigation on SpC. Capsaicin; In mucosal strips, SpC and tonic contraction were suppressed by $30 \mu\text{M}$ capsaicin and gradually recovered during wash-out (Figure 3). By contrast, SpC and tonic contraction of detrusor strips were increased by capsaicin. The effect on intact strips varied between strips but were less evident than on mucosa and detrusor strips. ***P2X₁ agonist***; Following 10 minutes' superfusion of $1 \mu\text{M}$ $\alpha\beta\text{mATP}$, AUC was increased, as a percentage of baseline, in detrusor (270% [190, 371]) and intact strips (344% [142, 441]) but was suppressed in mucosal strips (66% [33, 160]). $\alpha\beta\text{mATP}$ also generated a similar tonic contractions in intact ($28.9 \text{ mN} * \text{mm}^{-2} * \text{min}$ [20.1, 64.2]), detrusor (27.4 [9.3, 89.8]) and mucosal strips (20.0 [11.3, 34.2]). ***P2Y agonists***; effects of ADP (P2Y_1), UTP ($\text{P2Y}_{2,4}$) and UDP (P2Y_6) were investigated. Only UDP increased SpC in intact strips; ADP stimulated a tonic contraction in all three types of strips. ***Acidosis to pH 5.5 (MES buffer)***; SpC and tonic contractions in detrusor and intact strips were suppressed. Immediately after returning to pH 7.4, a large, transient tonic contraction was generated. A similar, but reduced, pattern was observed in mucosal strips.

Interpretation of results

The mucosal layer in guinea pig bladder generates baseline spontaneous contractions of comparable magnitude to detrusor, intact preparations generated significantly larger contractions. It is unlikely that the mucosa contractions are due to residual muscle as force normalised to muscle area was much greater than for detrusor. Moreover, mucosa and detrusor SpC had a different pharmacological profile to capsaicin and $\alpha\beta\text{mATP}$. We propose that different cellular components of these preparations generate spontaneous contractions. Tonic contractions are most likely generated by detrusor muscle. The data with UDP and the enhanced spontaneous contractions from intact preparations suggest an interaction between mucosa and detrusor.

Concluding message

Bladder mucosa is capable of generating spontaneous contractions independent of detrusor muscle, their response to exogenous agonists suggests they are generated by mechanisms different from those that contract detrusor smooth muscle.



References

1. Contractile properties of the pig bladder mucosa in response to neurokinin A: a role for myofibroblasts? Sadananda P, Chess-Williams R, Burcherv E. *Br J Pharmacol.* 2008 Apr;153(7):1465-73.

Disclosures

Funding: We thank Fukushima Medical University (Research Fellowship to Kushida N.), European Union; FP7 INComb and AgeUK (Research Fellowship to JSY) for financial support. **Clinical Trial:** No **Subjects:** ANIMAL **Species:** Guinea Pig **Ethics Committee:** the UK Animal Act 1986 and European Communities Council Directive 86/09/EEC