

## COMPARISON OF THE CONTRACTILE PROPERTIES OF MUCOSAL STRIPS FROM THE TRIGONE VS. THE DOME OF PIG URINARY BLADDER

### Hypothesis / aims of study

The mucosal layer of the bladder dome (urothelium, lamina propria and muscularis mucosa when present) has been demonstrated to develop spontaneous contractions (SCs) and respond to different pharmacological agents (1). Indeed, SCs of the bladder wall are reliant on an intact mucosa. When the two main regions of the bladder, the trigone and the dome are compared there is structural heterogeneity in the mucosal layer (2). Moreover, contractile differences between the detrusor muscle layers of the trigone and dome are well-documented. The trigone musculature itself exhibits significant spontaneous activity (3) and it has been suggested that this may aid contraction of the bladder neck during storage. However, the contractile properties of the trigone mucosal layer have not been studied and in particular whether they contribute to SCs in this region of the bladder. The aim of this study was to compare the basal SCs of mucosal layer of the trigone vs. dome of the porcine bladder and to investigate the effect of cholinergic agonist, carbachol (CCh), and a depolarising agent, KCl, on the contractile function.

### Study design, materials and methods

Pig (female, ≈6-months old) bladders were obtained from the local abattoir and transferred within one hour to the laboratory in ice-cold Krebs's solution. Strips of mucosa (separated from the detrusor by blunt dissection) from the dome and the trigone were mounted in organ baths, kept in carboxygenated Krebs' solution (pH 7.4±0.02) and maintained at 37°C. Isometric tension was measured, via UF1 force transducers connected to a Powerlab using Chart software. Basal SCs were recorded after 60 min of equilibration. Tonic contractile responses to 10 µM CCh and 80 mM KCl were also measured. The amplitude and frequency of basal SCs and the tonic contractile responses to KCl and CCh were compared using Student's unpaired t-tests. Data are presented as mean±SEM, *n*=number of separate preparations.

### Results

Mucosal strips from both the bladder dome and the trigone developed basal SCs. The amplitude of SCs in mucosal strips from the dome (*n*=17) was significantly (*p*<0.05) greater than the trigone (*n*=45) (dome 0.012±0.002 vs. trigone 0.007±0.001 g/mg tissue) (Figure 1A & 1B). However, there was no significant difference in the frequency of basal SCs between the dome vs. trigone (dome 22.8±1.1 vs. trigone 21.9±1.0 events in 5 mins) (Figure 1A & 1C). Stimulation of the mucosal strips with 10 µM CCh resulted in development of a tonic contraction in all strips. There was no significant difference in the magnitude of responses between the dome (*n*=20) vs. trigone (*n*=14) (dome 0.041±0.008 vs. trigone 0.035±0.008 g/mg tissue) (Figure 2A). Stimulation of the mucosal strips with 80 mM KCl also resulted in development of tonic contractions in all strips. There was no significant difference in the magnitude of responses between the dome (*n*=12) vs. trigone (*n*=10) (dome 0.054±0.012 vs. trigone 0.034±0.009 g/mg tissue) (Figure 2B).

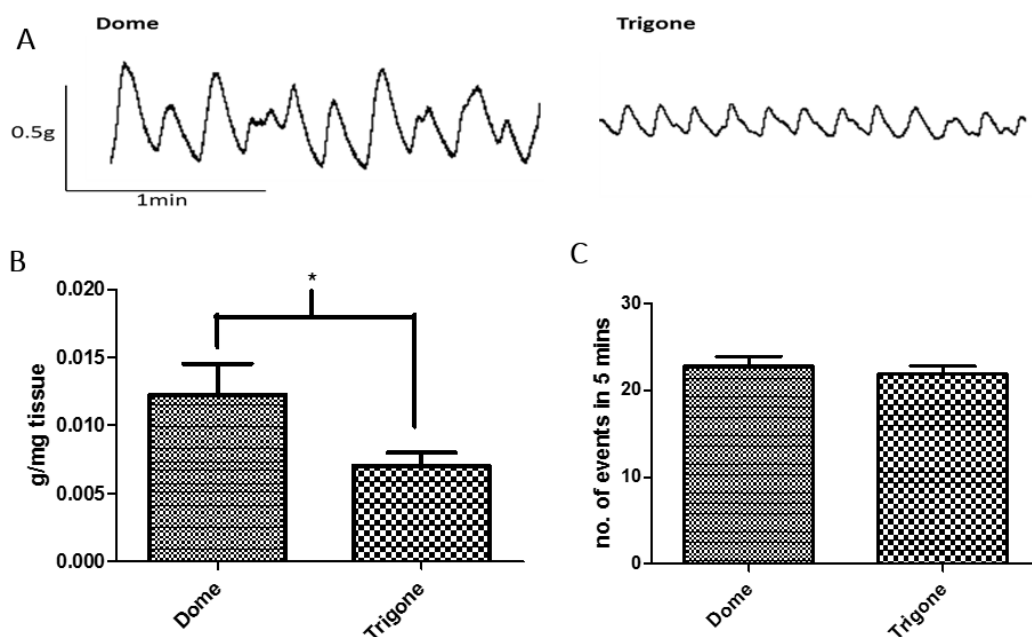


Figure 1) Comparison of the amplitude and frequency of basal SCs in mucosal strips from the trigone vs. dome. A) Representative chart traces of the basal SCs in the mucosal strips from the dome and trigone of pig bladder. B) The amplitude of SCs in mucosal strips from the dome (*n*=17) was significantly higher than those seen in the trigone (*n*=45). C) There was no significant difference in the frequency of basal SCs in mucosal strips of dome vs. trigone. Data are presented as mean±SEM (\**p*<0.05, unpaired t-test).

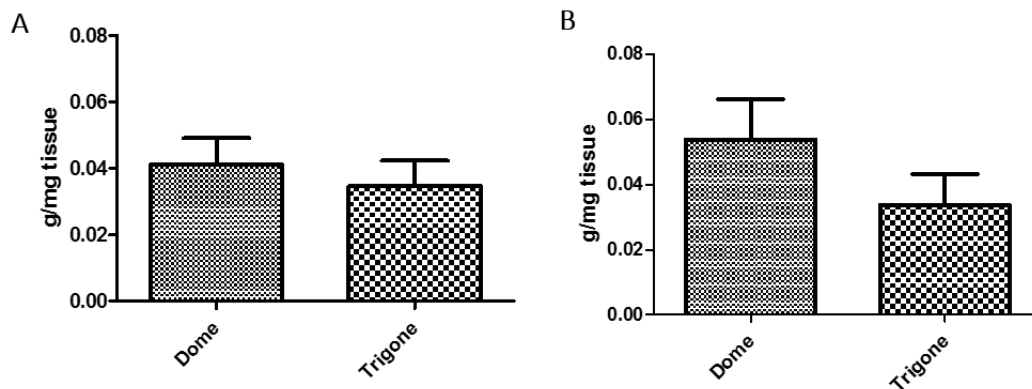


Figure 2) Tonic contractile responses of the mucosal strips from the trigone vs. dome of pig urinary bladder to A) 10  $\mu$ M CCh (trigone n=14, dome n=20) and B) 80 mM KCl (trigone n=8, dome n=10). Data are presented as mean $\pm$ SEM.

#### Interpretation of results

Basal SCs were recorded in mucosal strips from the pig bladder dome and trigone. The amplitude of basal SCs in mucosal strips from the dome was significantly larger than those from the trigone, demonstrating a functional heterogeneity between various bladder regions. Bladder mucosa from both regions contracted to a similar extent in response to cholinergic stimulation with CCh. However, mucosal strips from the dome were more responsive to KCl (although not significant). Recent studies have demonstrated a role of muscularis mucosa in generation and regulation of SCs in the guinea pig urinary bladder (2). This may also apply to the contractile responses to CCh and KCl in mucosal strips from both the dome and the trigone of pig urinary bladder.

#### Concluding message

Spontaneous phasic contractions were detected in pig trigone mucosa. Functional heterogeneity was seen between the mucosa of the dome vs. trigone with respect to the basal spontaneous contractions. The exact role of the SCs detected in the mucosal layer of trigone remains to be elucidated but they may play a role in regulating the SCs of the detrusor in this layer, contributing to the overall tone of the trigone. Mucosal strips from the trigone also contracted to CCh, similar to mucosal strips from the dome demonstrating the importance of cholinergic receptors in mediating the contractions of different regions of the bladder. Mucosal strips from both the trigone and dome regions also contracted in response to raised KCl, implying that the contractility of this tissue, like detrusor, is dependent on intracellular signalling and surface membrane pathways.

#### References

- 1) Andersson K.E & McCloskey KD (2014) Lamina Propria: The Functional Center of the Bladder? *NeuroUrol & Urodyn.* 33:9–16
- 2) Sanchez Freire V. et al. (2011) Structural differences between the bladder dome and trigone revealed by mRNA expression analysis of cold-cut biopsies. *BJU Int.* 108: E126–E135
- 3) Akino H et al (2008) Spontaneous contractions of the pig urinary bladder: the effect of ATP-sensitive potassium channels and the role of the mucosa. *BJU Int.* 102:1168-74

#### Disclosures

**Funding:** N/A **Clinical Trial:** No **Subjects:** ANIMAL **Species:** Pig **Ethics Committee:** No ethical approval was required as bladders were collected from the local abattoir after animals were killed for human consumption