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CHOLINERGIC MODULATION OF SPONTANEOUS CONTRACTIONS IN THE PIG BLADDER; THE ROLE OF THE MUCOSA IN THE DIFFERENT BLADDER REGIONS.

Hypothesis / aims of study

The structural and functional differences between the two main regions of the bladder, the dome and trigone, are well documented. The trigone musculature exhibits greater spontaneous activity than that of the dome (1) and it has been suggested that this may aid contraction of the bladder neck during storage. The mechanisms involved in generation and modulation of these spontaneous contractions (SCs) are however still not clear. It has been suggested that they may originate in the mucosal layer and may be modulated by release of transmitters such as acetylcholine from the urothelium. Recent studies have shown structural heterogeneity in the urothelium and suburothelium between the trigone, dome and lateral body (2), and functionally we have shown differences in the influence of the mucosa on basal SCs developed in isolated strips from these regions of the pig bladder (3). The aim of the present study was to investigate whether spontaneous contractions of pig bladder can be enhanced by cholinergic modulation, the influence of the mucosa, and any functional heterogeneity between the bladder regions.

Study design, materials and methods

Paired longitudinal strips were isolated from fresh pig bladders, from a local abattoir, from the 3 bladder regions - dome, body and trigone. The mucosa was carefully dissected from 1 strip per pair to produce intact and denuded strips. The strips were mounted in tissue baths in gassed Krebs bicarbonate buffer perfused with 95% O_2/5% CO_2 and maintained at 37^{o}C. Tissues were equilibrated for 60 minutes and SCs allowed to develop. Following equilibration the amplitude (peak tension per 5 min. period) and frequency (n^{o} of contractions per 5 min. period) of basal SCs were recorded. Tissues were then stimulated with low concentrations of carbachol (CCh) (0.01-0.1µM), 10 minutes incubation with each concentration and the effect of CCh on the amplitude and the frequency was recorded from the last 5 minutes with each concentration. Data are presented as mean ± SEM. Statistical analysis was performed via unpaired student t test to analyze paired strips of the same bladder region, whilst comparison between the 3 different regions was via one way ANOVA with a Bonferroni post hoc test.

Results

Isolated strips from dome, body and trigone all developed SCs when mounted in tissue baths and the time to development was similar in strips from all three regions as we have seen previously. Denuded strips of dome and body took significantly longer to develop SCs (dome: 40.5±9.9min, body: 44.4±11.8min, P<0.05), whilst denuded strips of trigone developed SCs in a similar time frame to intact strips (7.3±2.1min).

Basal SCs were significantly greater in amplitude in strips of trigone compared to dome and body (trigone: 0.004±0.0008g/mg strip weight, dome: 0.001±0.0001g/mg (P<0.01), body: 0.0011±0.0002g/mg (P<0.05), n=10), whilst frequency of SCs was similar in all three regions (dome: 10.2±2.7 in 5 min, body: 11.5±2.7 in 5 min, trigone: 5.0±1.5 in 5 min). Removal of the mucosa had no effect on the amplitude of SCs, but did reduce the frequency in strips dome and body (dome: 2.7±1.6 in 5 min, 3.4±1.1 in 5 min), but not in trigone (8.9±1.8 in 5 min).

The amplitude of SCs in intact strips was enhanced by low concentrations of CCh, especially in the trigone (Fig 1). Mucosa removal resulted in significant increase in amplitude of these SCs in the dome and trigone, with the body only showing significance at 0.05µM CCh (Fig 1). CCh also increased the frequency of SCs in the body but had little effect on the intact dome and trigone. In the dome, mucosa removal significantly increased frequency of SCs at 0.05 – 0.1µM CCh concentrations *p<0.05 (Fig 2) but not in the body or the trigone.

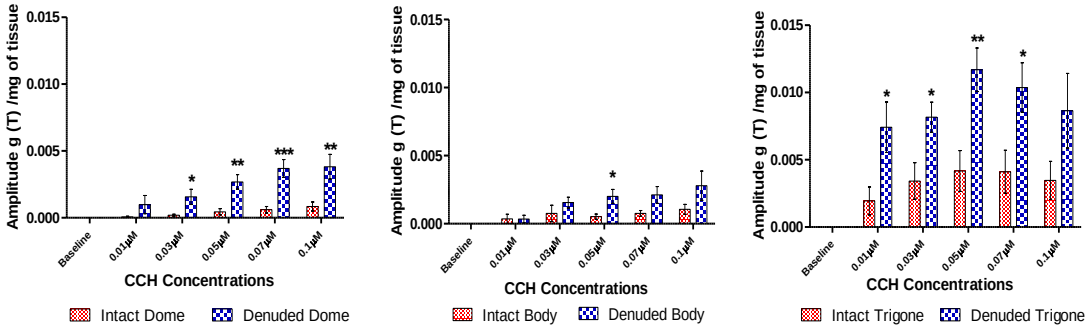

Figure 1 – Change in amplitude of SCs in comparison to baseline (0µM CCh) due to modulation by low concentrations of CCh in intact and denuded strips of pig bladder dome, body and trigone. Data are mean ± SEM; n=8 (dome and body), n=6 for trigone. P<0.05, **P<0.01 vs. intact.*

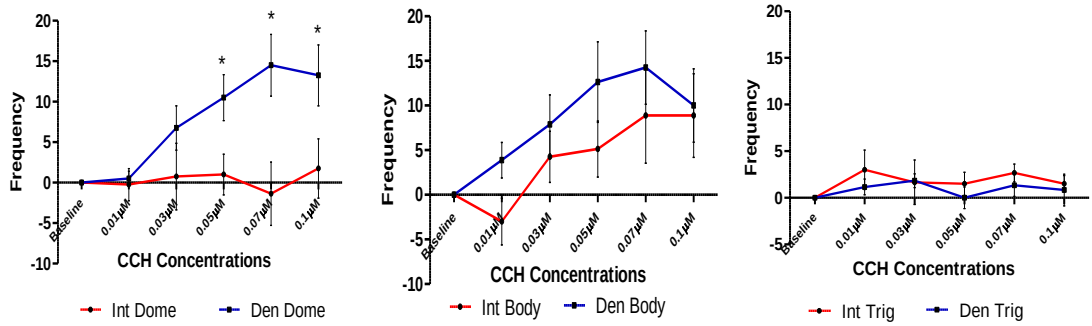

Figure 2 – Change in frequency of SCs in comparison to baseline due to modulation by low concentrations of CCh in intact and denuded strips of pig bladder dome, body and trigone. Data are mean ± SEM; n=8 (dome and body), n=6 for trigone. P<0.05 vs. intact.*

Interpretation of results

Spontaneous contractions seen in isolated bladder strips are greater in the trigone than in the dome and body, and although the mucosa can influence basal SCs in the dome and body it has little influence in the trigone. Low concentrations of carbachol have only a slight effect in increasing amplitude of SCs in intact strips of dome and body, but a greater effect in the trigone. Mucosa removal significantly enhances the amplitude of SCs in dome and trigone, but not the body. With regards to frequency, CCh modulation increased frequency in body and denuded dome but not the trigone and mucosa removal significantly enhances the frequency in dome and body with little effect on the trigone.

Concluding message

Spontaneous contractions of isolated pig bladder strips from dome, body and trigone can be modulated cholinergically. Functional heterogeneity is seen between the dome, body and trigone, with respect to the influence of the mucosa on basal spontaneous contractions and carbachol-stimulated spontaneous contractions. This may have implications for our understanding of bladder function.

References

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3. 3) Nyamwaro H, McKay NG, Lawson K, Chapple CR, Sellers DJ (2010) Phasic contractions of the pig bladder: Functional heterogeneity between bladder regions and the role of the mucosa. European Urology Supplements, 9(2): 123.

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Statement that no ethical approval was needed	**No ethical approval was required.**