166

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THE ROLE OF THE UROTHELIUM AND SUBUROTHELIUM IN SPONTANEOUS CONTRACTIONS OF PIG BLADDER DOME STRIPS

Hypothesis / aims of study
The mechanisms involved in the generation of spontaneous bladder contractions are still unknown, but may involve the urothelium and suburothelial myofibroblasts. This was investigated by examining strips of pig bladder dome of various thicknesses and investigating development of in vitro spontaneous contractions and the inhibitory effects of the potassium channel opener cromakalim.

Study design, materials and methods
Four types of pig bladder dome strips were examined (n=6 for each type). Strip A were fine strips composed only of urothelium/suburothelium. These were produced by removing detrusor muscle bundles meticulously via sharp dissection. Strip B were thin strips containing urothelium/suburothelium and some detrusor bundles. Strip C were intact strips and strip D were the denuded strips without urothelium/suburothelium. The histology of strips A and B was confirmed with immunofluorescence for alpha-smooth muscle actin (SMA) and vimentin.
The development of spontaneous contractions of each type of strip was observed in tissue-baths, and if spontaneous contractions developed then cumulative concentration response curves to cromakalim were obtained. The change in the frequency and amplitude of spontaneous contractions on addition of cromakalim was analysed. Drug-induced contractions of strip D to carbachol (1μM) and ATP (1mM) were also examined. A paired Student's t-test was used for statistical analysis, with a p-value of <0.05 considered statistically significant.

Results
Strip A was histologically confirmed to contain suburothelial myofibroblasts, (positive for both vimentin and alpha-SMA), but not detrusor muscle (positive for alpha-SMA, but negative for vimentin). Strip A did not show any spontaneous or drug-induced contractions to carbachol and ATP in the tissue-baths. Strip B was also histologically confirmed to contain suburothelial myofibroblasts and some detrusor bundles. Strip B, as well as C and D, showed spontaneous contractions. Cromakalim reduced the frequency of spontaneous contractions in these strips; pEC50s were 4.62 ± 0.25 (mean ± SEM), 5.34 ± 0.10, 6.01±0.14, in strip B, C and D, respectively (strip B and C vs. D, $p<0.01$ and <0.05, respectively). The amplitude of spontaneous contractions was also reduced by cromakalim and again the sensitivity to cromakalim was significantly higher in strip D, pEC50 in strip D was 6.13 ± 0.16, compared to 4.46 ± 0.80 and 4.12 ± 0.64 in strip B and C, respectively (strip B and C vs. D, $p<0.01$ in both).

Interpretation of results
Strips of urothelium/suburothelium which contain suburothelial myofibroblasts, but not smooth muscle bundles, do not contract when isolated in tissue-bath experiments. Strips containing urothelium/suburothelium and a few detrusor muscle bundles, do show spontaneous contractions, as do strip types C & D, the full intact and denuded strips. This implies that the detrusor muscle is necessary for the generation of spontaneous contractions. In the presence of the urothelium/suburothelium cromakalim was much less effective in reducing spontaneous contractions, which implies that these structures may have a role in regulation or modulation of spontaneous contractions. Interestingly, in full intact strips there appears to be a difference in the sensitivity to cromakalim in reducing the frequency of the contractions compared to the amplitude and role of the urothelium in this requires further investigation. If spontaneous contractions of bladder dome are under the control of the urothelium/suburothelium this may explain the phenomenon by which expansion of the bladder (storage of urine) enhances non-voiding contractions.

Concluding message
Detrusor smooth muscle is required for development of spontaneous contractions. The urothelium and suburothelium appear to be involved in regulation or modulation of these spontaneous contractions.

References
None

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