

CALCIUM SIGNALLING IN INTERSTITIAL CELLS OF CAJAL AND SMOOTH MUSCLE CELLS IN GUINEA-PIG BLADDER SHEETS

Hypothesis / aims of study

The bladder wall is a complex structure comprising several cellular types and related sub-populations. One cell type which has received significant attention over the last decade is the interstitial cell of Cajal (ICC), now identified in many smooth muscle preparations including tissues of the lower urinary tract (1). ICC exist as several sub-populations in the bladder wall: ICC-LP which are a network of interconnected stellate-shaped Kit and vimentin positive cells in the lamina propria region; ICC-IM which are non-networked elongated Kit/vimentin positive cells with lateral branches which track the detrusor smooth muscle bundles and ICC-IB which occupy the spaces between the detrusor muscle bundles (2). Physiological data on bladder ICC is accumulating and indicates differences in electrical and calcium signalling between ICC and smooth muscle cells (SMC).

The aim of the present study was to study calcium signalling patterns in bladder ICC sub-populations and bladder SMC in sheets of guinea-pig bladder.

Study design, materials and methods

Bladders were removed from male guinea-pigs (200-500g) which had been killed by cervical dislocation, opened longitudinally and the mucosa was removed from the underlying detrusor by sharp dissection. Preparations of mucosa were pinned urothelial surface down to a Sylgard recording chamber and loaded with the calcium indicator Fluo4 AM. The spontaneous activity that developed was recorded with an EMCCD camera imaging system.

Results

A number of cell populations were identifiable within these mucosal preparations, based on cellular morphology and distinguishably different calcium signalling patterns. These included detrusor and vascular smooth muscle and ICC of the detrusor (ICC-IM) and lamina propria (ICC-LP).

Detrusor smooth muscle displayed spontaneous increases in intracellular calcium at a mean frequency of 8.32/min (n=66), mean amplitude of 0.64 F/F₀ and mean duration of 2.69s. These short duration events were frequently recorded but longer lasting bursts of events were also encountered. These properties were different to that observed within vascular smooth muscle cells of mucosal preparations which displayed less frequent (1.5/min), longer lasting (8.92s) increases in intracellular calcium.

ICC populations displayed distinctly different signalling properties depending whether they were of the lamina propria or detrusor sub-types. Detrusor ICC fired more frequent (1.62/min, n=19), larger amplitude (1.52 F/F₀) signals than their ICC-LP counterparts which had mean frequency and amplitude of 1.34/min and 0.64 F/F₀ respectively (n=33). However ICC-LP increases in intracellular calcium were of significantly longer duration (23.5s) than those ICC found alongside detrusor smooth muscle (9.7s).

Interpretation of results

The findings of the present study have enabled us to characterize the calcium signalling patterns of detrusor smooth muscle, vascular smooth muscle and 2 subtypes of bladder ICC; ICC-LP and ICC-IM. It is clear that the frequency of detrusor smooth muscle calcium signalling is much higher than either the ICC-LP or the detrusor ICC and that the duration of ICC events is markedly longer than that of detrusor smooth muscle.

There are also clear differences in the calcium signalling properties of ICC-LP compared with detrusor ICC which is consistent with the view that these two populations have different physiological roles in bladder activity.

Concluding message

There are interesting differences in the calcium signalling properties of ICC-LP, ICC-IM and detrusor smooth muscle cells in whole sheets of guinea-pig bladder. These findings indicate the complex nature of signal integration in the bladder wall and highlight the need to consider the interactions of multiple cell types to fully understand the cellular basis of bladder filling and emptying.

References

1. Brading AF and McCloskey KD (2005) Nat Clin Pract Urol 2: 546-54
2. Davidson RA and McCloskey KD (2005) J Urol 173: 1385-90

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<i>Is this a clinical trial?</i>	No
<i>What were the subjects in the study?</i>	ANIMAL
<i>Were guidelines for care and use of laboratory animals followed or ethical committee approval obtained?</i>	Yes
<i>Name of ethics committee</i>	Guinea-pigs were sacrificed by cervical dislocation in accordance with Schedule 1 United Kingdom Animal Scientific Procedures Act (1986) and were approved by local University animal welfare and ethics committee.