

## SPONTANEOUS TRANSIENT OUTWARD CURRENTS IN INTERSTITIAL CELLS OF CAJAL ISOLATED FROM GUINEA-PIG BLADDER DETRUSOR.

### Hypothesis / aims of study

Interstitial cells of Cajal (ICC) have been found in many tissues of the lower urinary tract and there has been significant interest in this field over the last decade (ref). Bladder ICC fall into several sub-populations including lamina propria ICC and interbundle and intramuscular ICC within the detrusor smooth muscle. Electrophysiological studies of enzymatically dispersed detrusor ICC have shown they possess a complement of ion channels. Large-conductance calcium activated (BK), delayed rectifier and KCNQ voltage-dependent potassium channels have been characterized in detrusor ICC (1, 2).

ICC in several tissues exhibit spontaneous electrical activity as slow waves or spontaneous transient currents and the aim of the present study was to determine whether such events were present in detrusor ICC.

### Study design, materials and methods

Bladders were removed from male guinea-pigs (200-500g) which had been killed by cervical dislocation, opened longitudinally, pinned to a dissecting dish and the mucosa removed by sharp dissection. Small pieces of detrusor were incubated in collagenase/protease enzymes to release detrusor smooth muscle cells and ICC. ICC were selected for amphotericin perforated patch clamp experiments.

### Results

ICC were voltage-clamped at -60mV and stepped to a range of holding potentials up to +40mV in 20mV increments. ICC fired spontaneous transient outward currents (STOCs) at potentials positive to -60mV in physiological solutions (EK calculated to be -85mV). The reversal potential of the STOCs shifted as predicted by the Nernst equation when extracellular potassium was increased from 6mM to 60mM indicating that these were potassium conductances.

The identity of the ion channels responsible for the STOCs was examined using several pharmacological agents known to block BK currents. TEA (10mM, n=5), iberiotoxin (100-300nM, n=7 and n=10 respectively) and paxilline (100-300nM, n=7, 10 respectively) inhibited the amplitude and frequency of the STOCs and frequently caused a complete abolition.

The source of calcium responsible for the STOCs was then investigated. The role of calcium influx was examined by removing extracellular calcium (n=6) or by the L-type calcium channel blocker, nifedipine (1µM, n=6). Both of these interventions reduced the amplitude and frequency of the STOCs. Application of the ryanodine receptor inhibitor tetracaine (100µM) abolished the currents (n=11) whereas the phospholipase C inhibitor, 2.5 µM U-73122 (n=6) or 2-APB (30µM, n=4) decreased amplitude and frequency of the currents.

### Interpretation of results

Detrusor ICC from guinea-pig bladder fire STOCs which are caused by potassium ion flux through BK channels as demonstrated by their inhibition with BK channel blockers. Calcium influx and release from IP3 and ryanodine sensitive stores all appear to contribute to the spontaneous calcium events that trigger the STOCs.

### Concluding message

The findings of the present study demonstrate that guinea-pig detrusor ICC exhibit spontaneous transient outward currents which are mediated by BK channels. These currents typically oppose depolarization and lead to cellular hyperpolarization.

### References

1. McCloskey KD (2005) J Urol. 173: 296-301.
2. Anderson UA, Carson C, McCloskey KD (2009). J Urol. 182: 330-6

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<b>Is this a clinical trial?</b>	<b>No</b>
<b>What were the subjects in the study?</b>	<b>ANIMAL</b>
<b>Were guidelines for care and use of laboratory animals followed</b>	<b>Yes</b>

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*or ethical committee approval obtained?*

*Name of ethics committee*

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.

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