300

Bijos D[1], Drake M[1]
1. *University of Bristol, School of Physiology and Pharmacology*

SPREADING OF MICROMOTIONS IN JUVENILE RAT BLADDER: ORIGIN POINTS, DIRECTIONALITY AND FUNCTION OF GAP JUNCTIONS

Hypothesis / aims of study

Urinary bladders display spontaneous phasic activity manifested as pressure fluctuations and bladder wall movements i.e. micromotions. This phasic activity could be facilitated by cell-to-cell communication e.g. via gap junctions. During storage, phasic pressure changes are generally suppressed in a healthy bladder, but they appear in cystometric measurements in DO patients. An isolated organ model without neural control exhibits such phasic pressure fluctuations and micromotions. They optimize the bladder to accommodate increasing volume during storage, but increased phasic activity may result in pathologies. Bladders from young animals manifest phasic contractile activity, which changes during maturation and ageing. Understanding micromotions and pressure changes gives insight into factors moderating such activity, which could be relevant in the clinical context e.g. for overactive bladder syndrome.

If muscle cell cooperation happens due to cell communication via gap junctions, then blocking them might dampen the micromotions signal. Thus it is hypothesized that:

1) Gap junctions mediate propagation of movement and

2) Bladder wall motility does not necessarily generate change in intravesical pressure.

Hence, the aim was to characterize the effects of 18β-glycyrrhetinic acid (18β-GA), a known gap junction blocker, on whole organ pressure fluctuations and bladder wall micromotions, specifically looking at the relationship between micromotions and pressure changes.

Study design, materials and methods

Bladders were dissected from 3 week old (21+/-2 days) Wistar rats and catheterized with 26GA venflon (Becton Dickinson), filled with ~350µl Krebs (NaCl 118.4mM, glucose 11.7mM, $NaHCO_3$ 24.9mM, KCl 4.7mM, $CaCl_2$ 1.9mM, $MgSO_4$ 1.15mM, KH_2PO_4 1.15mM) and carbon particles were applied to the bladder surface to monitor movements. Bladders (n=15) were placed in an oxygenated organ bath (150ml) in cold Krebs solution. Pressure and video data were simultaneously acquired at 10Hz via LabView application (National Instruments, USA) and a camera (Prosilica EC650). Bath temperature was raised to 37°C. Pressure and movement were then inspected; if contractions were undetectable, 50-100µl was added to the intravesical volume. After at least 30min of equilibration in isovolumetric conditions, 30µM 18β-GA was administered (or 0.1% DMSO as a drug vehicle control). We compared 5 minute periods before and after drug addition using a two-tailed paired Student's t-test (GraphPad Prism) and calculated percentage change of each parameter. Distances between the carbon points on the bladder wall were analysed (LabView) and plotted in LabChart (ADInstruments).

Results

18β-GA decreases amplitude of phasic pressure changes in the whole organ

18β-GA decreased spontaneous phasic pressure fluctuations: the amplitude decreased by 79.8% 30min after addition of 30µM 18β-GA to the organ bath (N=6) ($p<0.001$). In half of the preparation gap junction blocker reversibly blocked phasic activity completely. In another half of preparations, when pressure fluctuations were detectable (>1cmH_2O amplitude), the frequency remained unchanged (3/6). Baseline was not affected.

Visual video inspection shows movement persists but pressure changes are obscured

Each bladder was observed during recording of the baseline activity and 30 minutes after addition of gap junction blocker 18β-GA. Movement of bladder wall persisted at 30 minutes after addition of 18β-GA in all preparations, including three experiments without detectable pressure changes (<1cmH_2O).

Distances on the bladder wall with a substantial longitudinal component (Fig .1A) elongated and shortened, which coincided with pressure fluctuation changes (Fig.1B, arrows). However not all pressure changes correlated with visible movement. In Fig.1B only every other pressure fluctuation could be explained by localized bladder movement, suggesting local contractions in other bladder areas influenced pressure change.

Quantification of distances between points on the bladder wall confirmed that blocking gap junctions with 30µM 18β-GA alters micromotions. Although 18β-GA abolished pressure fluctuations completely (N=3), movement was present. In Fig.1C longitudinal distance number 2 was shortening despite the lack of visible pressure change.

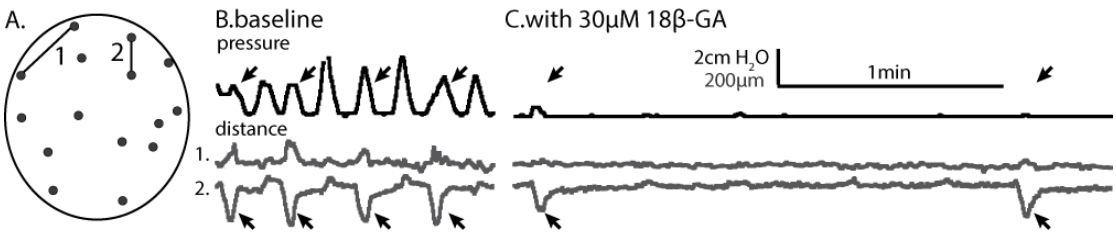

Figure 1. Effect of gap junction blocker on pressure and micromotions. A) Schematic of the bladder wall surface filmed with black carbon particles added to facilitate movement analysis. Pressure and distance traces B) before and C) after addition of 18β-GA.

Spreading movement: We measured the time difference between the peak pressure measurement and the maximum shortening of the microcontraction. We followed consecutive longitudinal and transverse distances in the spontaneously contracting bladder

(Fig.2). In the measured area the movement was happening mostly after the pressure change. Longitudinal distances showed a dynamic pattern of contractions, where one part was contracting before the other (Fig.2C, distance 1 contracts before distance 2). Transverse distances were contracting at the same time (Fig.2D).

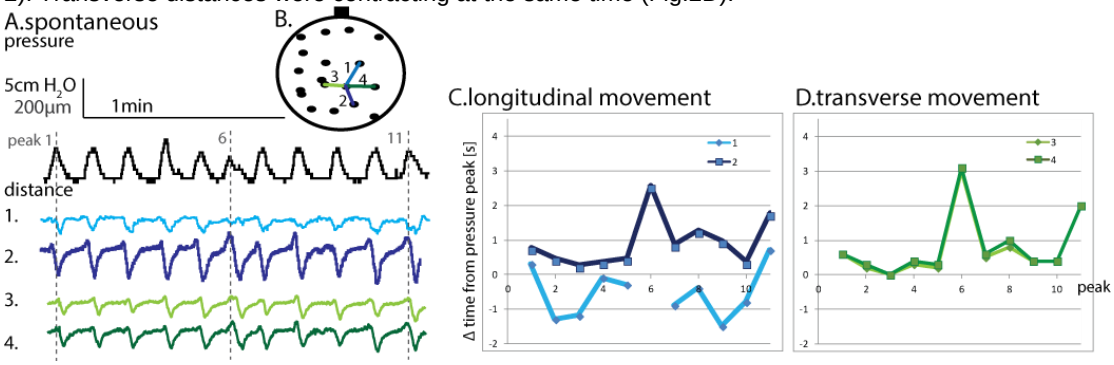

Figure 2. Spreading of micromotions along the bladder wall during spontaneous contractions. A) Pressure and distance traces, B) schematic of the bladder and the position of distances. Time difference (in seconds) between the peak pressure measurement and the maximum shortening of the microcontraction in C) longitudinal and D) transverse direction on the bladder wall.

Interpretation of results

Microcontractions and microelongations of the bladder wall possessed rhythmicity which was not always synchronous with pressure change. This suggests that rhythmical microcontractions on the bladder wall are independent of pressure fluctuation and might not necessarily cause a change in bladder pressure. It might still however be relevant for afferent signalling and sensation of the bladder.

Longitudinal spreading of movement most likely relates to the longitudinal positioning of muscle fibers and thus affects spreading of contractions. In general, in spontaneously active whole bladder the movement initiation centres were localized at the top of the bladder dome and bladder base (in superior and/or inferior sections of the bladder) and spreading vertically (longitudinally).

Although micromotions decrease with a decrease in pressure fluctuations, the initiation of the bladder micromotions is independent of gap junction communication.

Concluding message

We confirmed that bladder wall motility does not necessarily generate change in intravesical pressure. Gap junctions change but do not block bladder wall movements.

Disclosures

Funding: Marie Curie Actions "Training Urology Scientists" grant **Clinical Trial:** No **Subjects:** ANIMAL **Species:** rat **Ethics Committee:** UK regulations