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EFFECT OF ISOPRENALINE ON MICROMOTIONS AND PHASIC PRESSURE FLUCTUATIONS IN JUVENILE RAT ISOLATED BLADDER

Hypothesis / aims of study

Bladder micromotions are localized and propagating movements, increasing in intensity with increasing bladder volume, similar to non-voiding contractions observed in cystometric studies [1]. Their origin and function is not understood, but they are suggested to be involved in pathological states like urgency.

In the unstimulated whole organ, a proportion of the bladder is motile due to initiation and propagation of contraction. However, propagation of motility covers only part of the entire organ, meaning that there are also non-motile areas [2]. These non-motile areas are likely to be important for the urodynamic effects of motility on intravesical pressure. For example, if the “tone” of non-motile areas is low, they are likely to dissipate the force of the contractions in the motile areas, such that they have only small effects on intravesical pressure.

It is impossible to characterise the influence of non-motile areas on bladder pressure from conventional muscle strip experiments. Thus we employed a whole organ approach to model pressure changes under conditions replicating the storage phase of the micturition cycle, and evaluated motile and non-motile parts of the bladder during micromotions.

Isoprenaline stimulates beta-adrenoceptors on bladder smooth muscle, causing relaxation. This was used to assess whether motile areas (reduced frequency and/or amplitude of pressure fluctuations), and/or non-motile areas (elongation) were affected. To determine how movement and pressure fluctuations are related we used an ex vivo organ bath approach with simultaneous filming of the bladder surface.

Assuming that contraction is responsible for pressure we hypothesize that movement and pressure vary independently and aim to investigate the interplay between motile and non-motile areas in the micromotions of the bladder wall in a spontaneously active bladder exposed to isoprenaline.

Study design, materials and methods

3 week old (21+/- 2 days) Wistar rats were killed by UK Schedule 1 procedures (N=7). The abdomen was opened, intestines were retracted and the ureters were cut. The pubic bone was cut open, and the bladder and the urethra were gently dissected and placed in cold Krebs buffer (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). A 26GA venflon (Becton Dickinson) attached to a 1ml syringe filled with Krebs was inserted into the bladder via the urethra and secured with a thread above the vesico-ureteric junction. The bladders were filled slowly up to ~350µl and carbon particles were applied to the bladder surface to allow monitoring surface movements. The bladder was then placed in cold Krebs solution in an oxygenated bath with an optical window for filming. A camera (Prosilica EC650) connected to the custom written LabView application (National Instruments, USA) allowed simultaneous acquisition of pressure and video data at 10 frames per second. Bath temperature was raised to 37°C and an initial equilibration of the bladder in the bath took minimum 15min. 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 condition, drugs were administered. 1, 10, 100 and 1000 nanomolar (nM) isoprenaline (Sigma, UK) in 10µM ascorbic acid (to prevent oxidation) was added into the bath.

We analyzed three parameters of bladder pressure traces: amplitude, frequency and baseline of spontaneous phasic contractions. 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 after drug addition. Distances between the carbon points in the focused field of view on the bladder wall were analysed using an in-house custom LabView application and plotted in LabChart (ADInstruments).

Results

Isoprenaline reversibly reduced the amplitude of spontaneous pressure fluctuations in a dose dependent manner. 100nM and 1uM isoprenaline abolished spontaneous pressure fluctuations completely ($p<0.001$) in a reversible manner within 30s of addition of the drug to the bath. 1 and 10nM isoprenaline caused a reduction in spontaneous pressure fluctuation amplitude by 10.6±7% ($p<0.05$) and 34.4±5% ($p<0.01$), respectively, without significantly affecting frequency. All doses reduced the baseline pressure of the bladder. 1nM reduced baseline by 4.9±2% ($p=0.079$), 10nM by 4.4±1% ($p<0.05$), 100nM by 11.1±1% ($p<0.001$) and 1µM by 14.7±4% ($p<0.001$). Ascorbic acid alone had no influence on bladder pressure parameters.

Simultaneous visual inspection of video recordings and the pressure trace showed that each pressure fluctuation is a composite net effect of bladder wall motility. Bladder wall movements are made up of parts of the bladder contracting (Fig 1A upper distance trace) and other areas elongating (Fig 1A lower distance trace). With 100nM and 1µM isoprenaline, all movement was abolished. At lower doses we found that as pressure baseline decreased (Fig1B, pressure trace) parts of the bladder stopped contracting (shortening) and started to elongate (Fig1B distance trace).

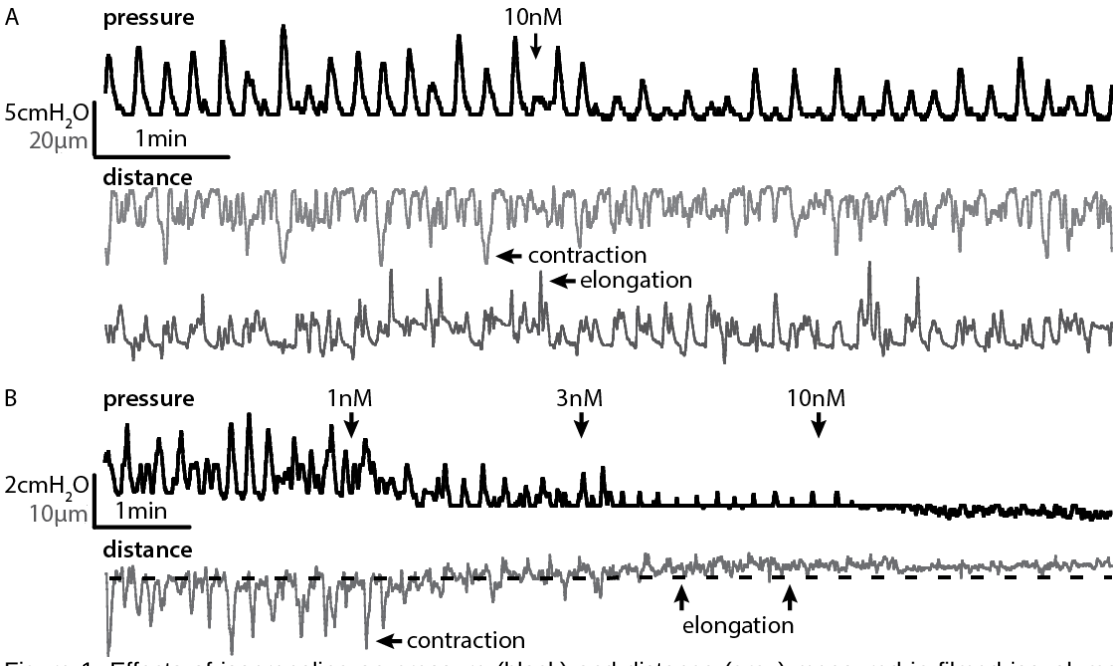

Figure 1. Effects of isoprenaline on pressure (black) and distance (grey) measured in filmed isovolumetric ev-vivo juvenile rat bladder preparation. Distances are measured between two points on the bladder wall. A) Two independent distances demonstrate an actively contracting area and an elongating (relaxing) area. B) Dose dependent decrease of pressure baseline causes inhibition of shortening (contraction) of the distance and overall elongation (increase in distance - above the dashed line).

Interpretation of results

Fluctuations in distances show that the intravesical pressure results from net summation of movements, some relaxing and some contracting. It also suggests that the bladder is a modular structure and that during micromotions motile and non-motile areas are not fully coordinated. Baseline pressure drop is a net result from increased elongation of parts of the bladder and decreased motility.

Concluding message

Low doses of isoprenaline relax the ex vivo iso-volumetric whole organ bladder preparation. Quantification of distances on bladder wall allows inspection of motile and non-motile areas and shows that pressure trace is a composite of contractile and relaxing movements in the bladder wall.

References

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Disclosures

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