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Nishikawa N[1], Jabr R[2], Wagg A[3], Fry C[1]
1. *Department of Physiology and Pharmacology, University of Bristol, UK,* ***2.*** *Faculty of Health and Medical Sciences University of Surrey, UK,* ***3.*** *Faculty of Medicine & Dentistry University of Alberta Canada*

AGEING, BLADDER STIFFNESS AND ATP RELEASE, A PATHOPHYSIOLOGICAL BASIS OF URGENCY.

Hypothesis / aims of study

Mechanical impacts on the bladder wall, such as lateral stretch or increased transmural pressure releases ATP, among other modulators, that is proposed to activate bladder afferents. This provides a sensory transduction system to measure the extent of bladder filling. Several reports have shown augmented ATP release from bladders of ageing animals and humans, or from bladders with pathologies associated with increased urgency. However, the underlying mechanisms whereby external mechanical interventions elicit ATP release and how this changes with age and bladder pathologies is unknown. We have investigated the hypothesis that mechanical stiffness (stress per unit length change) of the bladder wall changes with age, or following obstruction, and that stress (tension) generated in the bladder wall during mechanical stretch, rather than the magnitude of stretch itself, is the determinant of the quantity of ATP release. We have tested the hypothesis in vitro using human and animal bladder wall tissue of different ages and pathologies.

Study design, materials and methods

Human detrusor biopsies were obtained from patients undergoing either: cystectomy but with symptomatically normal bladder function (n=10; 6 men, 4 female, 60.4±12.7 yr); or alleviation of outflow tract obstruction (n=11; all male; 65.5±11.0 yr). Mouse detrusor was obtained from bladders of C57/BL6 mice aged 12 weeks or two years. Detrusor strips 0.5-0.9 mm diameter, 5 mm length were (mucosa-free for human preparations) tied between a hook and an isometric force transducer and superfused with Tyrode's solution (including 24 mM $NaHCO_3$, 5% CO_2, pH 7.40 ±0.02, 37'C). The hook was connected to a voltage-activated solenoid that allowed rapid (<20 µs) extension of the muscle to a new length and equally rapid restoration of the original length after 60-100 s. The increase of steady-state tension upon stretch (T_{ss}) was measured after 60 seconds. Extensions of the preparation were to 104, 108, 112, 116 and 120% of the original length and T_{ss} was measured as a function of extension. The slope of the tension/extension (stress/strain) relation is stiffness was tested for linearity. After one-hour rest at resting length to allow releasable ATP pools to be restored, as previously determined, a subsequent experimental extension to 112% of the resting length was done to measure T_{ss} and associated ATP release. Units of stiffness were MPa ($N.mm^{-2}$ cross section area). Superfusate samples (20 µl) from mouse preparations were taken immediately above the preparation at 0.1 minutes after rapid stretch and immediately frozen. ATP concentration was measured with a luciferin-luciferase assay (FL-AA, Sigma Aldrich). The luminometer was calibrated on each occasion with ATP standards (0.1–1000 pM) in Tyrode's, and with Tyrode's as a blank solution. Data are medians [25,75% interquartiles]. Unpaired data set comparisons used ANOVA and *post hoc* non-parametric tests (n, number of preparations) with Bonferroni corrections. Spearman's coefficients, r, expressed associations between variables with estimation of p (vassarstats.net /tabs_r.html). The null hypothesis was rejected at p<0.05. For each experiment n≥8, based on power calculations(80% power, alpha=0.05) from previous similar experiments.

Results

Passive muscle stiffness. With human samples from stable and obstructed bladders there was a significant, age-dependent increase of passive stiffness in each cohort (Figure 1). However, the age-corrected (60 years) stiffness was similar in both groups (0.091±0.078 and 0.156±0.083 MPa, p>0.05, n=10,11 respectively). This was matched by an increase of stiffness in the old mouse cohort in comparison to the young group (Figure 2). However, mouse tissue stiffness values were significantly greater than for either human stable or obstructed bladder tissue, with the caveat that age-matching was not possible (e.g. 0.66±0.31 vs 0.17±0.13 MPa, young mouse vs stable human bladder, p<0.05).

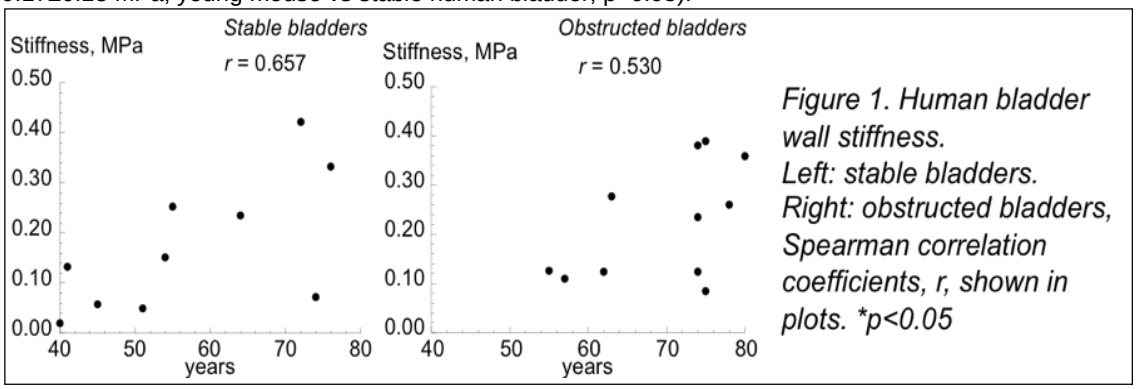

*Figure 1. Human bladder wall stiffness. Left: stable bladders. Right: obstructed bladders, Spearman correlation coefficients, r, shown in plots. *p<0.05*

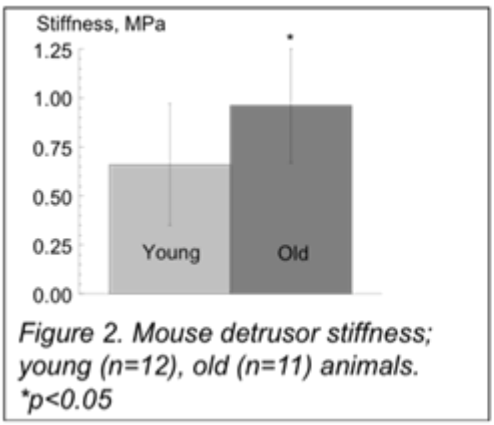

*Figure 2. Mouse detrusor stiffness; young (n=12), old (n=11) animals. *p<0.05*

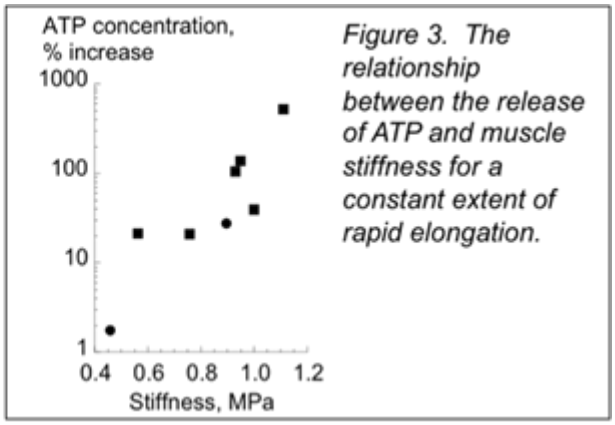

Figure 3. The relationship between the release of ATP and muscle stiffness for a constant extent of rapid elongation.

ATP release and bladder wall stretch, mouse tissue. Stretch-induced ATP release was measured in mouse preparations as they had an intact mucosa, from where such release occurs. The range of tissue weights was small (16.4 [15.2, 16.9] mg) and there was no relation with pre- or post-stretch ATP levels. Resting (pre-stretch) ATP concentration was 1.43 [0.30, 4.53] pM, *n*=8. Stretch by 12% of resting length increased the ATP concentration by a median of 33.5 [21.2, 113.2]%. However, there was a significant (*r*=0.878), positive association between the percentage increase of ATP concentration and preparation stiffness (Figure 3, note also the logarithmic axis of the ordinate). Data are shown for old animals (squares) and young animals (circles). For mouse stiffness values (Fig 2) an increase of median stiffness from 0.66 to 0.96 MPa with age is associated with a 3.7-fold increase of ATP release.

Interpretation of results

Ageing is associated with increased passive muscle stiffness. This was present in human and mouse detrusor, moreover with the human data absolute values and the ageing trend was observed in functionally normal bladders and those with outflow tract resistance. With mouse bladders, where measurement of stiffness and ATP release was simultaneously recorded, stretch-induced ATP release was significantly associated with tissue stiffness. We have shown for the first time that when the bladder wall is stretched during filling it is the tension generated in the wall that induces ATP release and not the degree of stretch *per se*. The transducing element that links wall tension to mucosal ATP release remains to be characterised.

Concluding message

The increase of urinary urgency with ageing may be explained by increased stiffness of the bladder, possibly due to deposition of more extracellular matrix, and linked to increased ATP release.

Disclosures

Funding: Nobuyuki Nishikawa got a financial support from the Astellas Foundation for Research on Metabolic Disorders and the Braithwaite Family Foundation. Christopher Fry got an NIH grant: NIH 1R01DK098361. **Clinical Trial:** No **Subjects:** HUMAN **Ethics Committee:** Biopsies were collected with local ethical committee(University College London Hospitals ethics committee, and the experiments were done at University College London) approval and written, informed patient consent. Mouse bladders were removed after euthanisation according to UK Home Office schedules governed by the Animals Act, 2013 amendments. **Helsinki:** Yes **Informed Consent:** Yes