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SIMULTANEOUS MULTI-POSITION URETHRAL BIOMECHANICS: THE EFFECTS OF DIABETES ON URETHRAL BIOMECHANICAL PROPERTIES IN THE RAT

Hypothesis / aims of study

The physiological function of any tissue is ultimately limited by its biomechanical properties. As such, any changes in tissue biomechanical properties with disease are likely to adversely affect function. While a great deal of attention has been paid to the biomechanical properties of the urinary bladder, relatively little exploration has been directed toward understanding urethral biomechanics. Given the obvious importance of the urethral outlet in both storage and voiding, it is essential to understand the biomechanical limits of this portion of the lower urinary tract. Prior studies of urethral ex vivo biomechanical properties in health and disease have utilized measurement devices limited to measurements at a single point along the longitudinal axis. We have developed a technique which allows simultaneous measurement of urethral diameter along multiple longitudinal axis positions. This reduces the number of animals required to investigate regional differences and allows for enhanced statistical power.

Diabetes mellitus (DM) affects ~285 million people worldwide and has dramatic effects on lower urinary tract function, and it has also been determined to be a high risk factor for urinary incontinence (UI). In the early stages, it appears to result in bladder overactivity, while in later stages of disease, diabetic cystopathy is characterized by bladder underactivity. Changes in urethral biomechanical properties could certainly impact either early or late stage changes in bladder function, such that decreases in outlet resistance due to increases in compliance might result in UI while increases in outlet resistance due to decreases in compliance would favour retention.

In order to better understand the impact of diabetes on urethral function, we examined the effects of chronic diabetes on urethral compliance in both contracted and passive states in rats.

Study design, materials and methods

As a first step in determining the effect of DM on urethral function using our newly developed biomechanical methodolgy, we administered streptozotocin (65 mg/kg) or vehicle to female Charles River Sprague-Dawley (SD) rats (n=4-5/group). Rats were tested weekly for blood glucose and those with circulating levels of ≥300 mg/dl were considered DM. Ten weeks later, control and DM animals were sacrificed and their urethras were harvested and mounted at in vivo length to stainless steel tubes in a chamber of oxygenated Krebs at 37C. The urethral lumen were connected, via the stainless steel tubes, to a fluid filled reservoir on a vertical post in order to adjust intraluminal pressure from 0-20 cmH_2O. Following drug treatments, changes in urethral diameters were measured as pressures were stepped in an escalating fashion for 60 seconds at each step. Video capture with edge detection allowed for simultaneous recording of urethral diameter at multiple points along the urethral length. The distal tie position was designated as p0, the proximal tie as p100, and intervening 9 equally spaced distances along the length of the urethra were used for diameter measurements. Measurements were made under conditions of contraction (bath application of 100 uM L-NO-Arg and 40 uM phenylephrine; contracted state) and following complete relaxation by addition of EDTA (3 mM; passive state). Compliance was estimated using the change in diameter at each pressure step.

Results

Urethral compliance was bimodal, with peaks at p30-40 distal positions, and at p90 proximal position. The middle urethral positions of p50-60 were relatively low compliance regions. This bimodality was lost in the contracted condition of control urethras at 10 cmH_2O and above, demonstrating a relatively strong active contraction of the distal urethra, but not of the proximal urethra.

In contracted control urethras, both pressure and position were non-interacting significant factors ($P<0.0001$ for both), with significant differences from D_0 by 12 cmH_2O and higher at proximal positions p80 and p90 (~30-40%). Plateau of effect was seen at 2 cmH_2O (comparisons of D_2 to D_{4-20} were not significant by post-test). Following EDTA, both pressure and position were also non-interacting significant factors ($P<0.0001$ for both), and significant differences from D_0 were seen by 4 cmH_2O in all but the p50 position. By 6 cmH_2O, all positions were greater diameter than D_0. Plateau of effect was seen at 8 cmH_2O (D_8 post-test comparisons to D_{10-20} not significantly different).

In the contracted state, DM urethras maintained their bimodal appearance with increasing pressures up to and including 20 cmH_2O. Pressure and position interacted ($P=0.0030$ for interaction, $P<0.0001$ for each independently). In this case (as opposed to contracted state of the controls), changes from D_0 were seen as early as 6 cmH_2O and plateau was not seen until 12 cmH_2O (greater than both the contracted and passive control states). In the passive state, both pressure and position were non-interacting significant factors ($P<0.0001$ for both), and significant differences from D_0 were seen by 4 cmH_2O. Plateau of effect was seen at 4-6 cmH_2O (D_4 post-test comparisons to D_{6-20} not significantly different except for p90 at 20 cmH_2O, while complete plateau from D_{6-20}).

When compared to controls, urethras from DM rats demonstrated an overall increase in compliance in both the contracted ($P<0.0001$ for interaction, $P<0.0001$ for DM and pressure) and passive ($P=0.0096$ for interaction, $P<0.0001$ for DM and pressure) states, and these differences were significantly different from 10-20 cmH_2O in both conditions. DM rats had a significantly greater compliance along the longitudinal axis of the urethra in both the contracted (no interaction, $P<0.0001$ for DM and $P=0.0016$ for position) and passive (no interaction, $P<0.0001$ for DM and $P=0.0044$ for position) states, with the distal region p30 being the most affected (significantly higher compliance in DM at this position by post-test).

Interpretation of results

In the current study, we have demonstrated an increase in urethral circumferential compliance with 10 weeks of DM. We have also observed a weaker contractile response (i.e. decrease in compliance) to pharmacological challenge in these whole mounted DM urethras. Such results are important when considering the relationship between DM and UI, as this set of conditions might predispose DM patients to stress UI. Previous work using a different methodology and a different strain of rat

showed a decrease in urethral compliance with 10 weeks of DM. It is tempting to speculate that the difference in results between this and the previous study is, in fact, the interaction of DM with genetic background. Future studies should examine whether this increase in compliance is transient and develops into decreases in compliance, or if it extends into end stage cystopathy.
We have also seen a mid-urethral (p50-60) region of low compliance, which likely corresponds to the mid-urethral high pressure zone found in rat urethral profilometry. We suggest that this region may be that of the thickened band external urethral sphincter, and as such acts as a mechanical stricture with respect to the challenges of these studies. This is again in contrast to earlier work done with a different methodology where a proximal to distal gradient was seen in passive urethral compliance. Once again, here we utilize the entire length of the urethra from the same rats (longitudinal approach), rather than making comparisons across positions using different animals for each position (cross-sectional approach). Clearly, more work is required to resolve discrepancies.

Concluding message

The current finding of increases in urethral compliance in both contracted and passive states following 10 weeks of diabetes in rats might help explain the strong association with DM and UI (DM women are 50-200% more likely to have UI). Further studies are necessary to confirm the suspected progression from increased urethral compliance of early stage disease progression to that of decreased compliance which might be associated with end stage disease.

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