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URETHRAL BIOMECHANICS IN HEALTHY AND DIABETIC FEMALE RATS

Aims of Study: Proper function of the urethral outlet is important for normal voiding, yet many of the properties of the urethra remain unexplored. In particular, little is known about urethral biomechanical properties under normal or pathological conditions. We have performed biomechanical studies on whole-mounted rat urethras of normal and diabetic female rats, including measures of compliance and responses to pharmacological challenges.

Methods: A transurethral catheter (PE10) was inserted in halothane anaesthetized normal and 3, 5 and 10-week streptozotocin-induced diabetic mellitus (DM) female S-D rats (250-300 g) and the lower urinary tract was exposed via a midline abdominal incision. A cystotomy was made at the apex of the bladder dome and the catheter exited. The in vivo length of the urethra was maintained by secure ligation at the distal-most end of the urethra and mid-bladder. The ureters were tied and served as landmarks. The urinary tract was carefully dissected free of surrounding tissues, transferred to oxygenated medium and mounted in a laser micrometer apparatus, originally designed for vascular biomechanics, at in vivo length. The medium was continuously oxygenated and maintained at 37C. Measurements of pressure-diameter relationships were made at 30% (proximal urethra), 50% (middle urethra) and 70% (distal urethra) of the in vivo length using stepwise increases in intraurethral pressure from 0-20 mm Hg for measures of compliance. In urethras from normal rats, the urethromotor responses of smooth or striated muscle were made at the middle urethra by addition of L-NO-Arg (100 μM) for NO synthase inhibition followed by phenylephrine (PE, 40 μM) for smooth muscle responses or acetylcholine (ACh, 10 mM) following atropine pre-treatment (1 μM) and/or hexamethonium (HEX, 100 μM) for striated muscle responses. Following these treatments, 1.5 mM EDTA was added to the bath.

Results: Control urethras are characterized by a proximal-distal compliance gradient (80, 60 and 20% diameter change at highest intraluminal pressure, 25 mm Hg, for proximal, middle and distal regions, respectively). There were no differences seen between control and 3 weeks DM, nor were there any changes in distal region compliance with any DM time point. However, a dramatic and progressive decrease in compliance was seen in the proximal and middle urethral regions, such that by 10 weeks DM, both proximal and middle region compliances had decreased to values equivalent to the distal region. Urethromotor responses at a clamped intraluminal pressure (IP) of 8 mm Hg (the EP_{50}, resulting in an ~20% increase in urethral diameter) in control urethras revealed a gradual contraction of 32% following addition of L-NO-Arg, and a rapid contraction following PE to diameters indistinguishable to those at 0 mm Hg IP, and these contractile responses were reversed to greater than control values by EDTA (~25% increase in diameter). For urethromotor responses due to striated muscle contraction, atropine had no effect, while Ach resulted in a transient 30% contraction followed by a sustained 15% contraction. As seen before, addition of EDTA resulted in diameters exceeding control values (27%). HEX pre-treatment resulted in an abolition of the sustained response to ACh, suggesting that this long lasting contraction may be due to presynaptic neuronal nicotinic receptor activation possibly favouring norepinephrine release at the level of the smooth muscle. Studies are currently underway to dissect the specifics of this interesting phenomenon.

Conclusions: We have demonstrated in the first ever, *ex vivo* whole-mounted urethral preparation that a proximal-distal gradient in compliance occurs in normal urethras, and that DM results in a collapse of this gradient favouring stiffening of the more proximal regions to equivalent low compliance of the more distal region. This has obvious clinical importance with regard to the voiding dysfunction of DM, which is generally attributed entirely to diabetic cystopathy. At the end stages of diabetic cystopathy, the hypomotile bladder is forced to contract against an outlet with increased resistance, a condition that results in a positive-feedback cycle of detrusor decompensation. Moreover, we have demonstrated that this preparation allows for pharmacological evaluation of urethral function, and as this work is yet done at the middle region of normal animals, the technique will allow us to determine regional differences in urethromotor responses in the urethras from normal, surgically altered and diseased animals.