Development of an experimental system for the study of urethral biomechanical function

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Jankowski, Ron J., Rachelle L. Prantil, Matthew O. Fraser, Michael B. Chancellor, William C. de Groat, Johnny Huard, and David A. Vorp. Development of an experimental system for the study of urethral biomechanical function. Am J Physiol Renal Physiol 286: F225–F232, 2004. First published September 23, 2003; 10.1152/ajprenal.00126.2003.—Despite its principal mechanical function in the storage and release of urine, the biomechanical properties of the urethra have remained largely unexplored. The purpose of this study was to develop and validate an experimental model that can be used for evaluating whole urethral tissue in such a manner. Bladder-urethral specimens were excised from halothane-anesthetized female rats and mounted at in vivo length within the experimental apparatus consisting of a tissue perfusion chamber, an adjustable fluid column, and a laser micrometer. Outer diameter measurements were made at proximal, mid, and distal axial locations in response to increases in intraluminal pressure and after addition of various muscle-responsive agents. Basal smooth muscle tone and regional variations in compliance were detected through pressure-diameter responses. Chemically evoked contractile responses were measured and correspond to regional compositions of intrinsic smooth and striated muscle components. The results presented illustrate the utility of this system, which should permit a more thorough characterization of structure-function relationships and urethral biomechanical function in relation to normal and dysfunctional tissue states.

urethra; smooth; striated; muscle; biomechanics

THE ABILITY TO PROPERLY CHARACTERIZE fundamental mechanical tissue properties can be very powerful in understanding normal tissue function, as well as in elucidating underlying causes of dysfunction and the pathological progression of disease states. The urethra, which obtains its mechanical properties from both passive connective tissue elements and active smooth and striated muscle elements, functions as both a mechanical barrier to urine leakage during bladder filling and as a conduit for its release during micturition. Surprisingly, however, few studies have attempted to characterize the mechanical behavior of this tissue to date (4, 15, 23). This may be attributed, at least in part, to the lack of appropriate experimental systems to perform these kinds of investigations. Ex vivo experimental systems have been extensively utilized to examine the relationship between microstructure and mechanical function of vascular segments and have assisted in the development of working hypotheses regarding the initiation and progression of vascular diseases (10, 26–28). Application of similar experimental devices to the urethra could yield similar success.

Urodynamic studies have previously been performed to examine the relative contributions of the various urethral components to normal closure and their respective roles in the development of incontinence (1). However, the anatomic basis for these physiological studies is still unclear because, to date, the mechanical contributions of the various components of the urethra have yet to be clearly defined (19). Our goal for the current study was to develop a system that can be used for the characterization of both active and passive mechanical behavior of the isolated urethra, thus enhancing the understanding of the structure-function relationship of the individual components comprising this tissue.

We describe here a whole-mounted ex vivo urethral testing apparatus and preparation that can be used to study regional urethral behavioral responses in terms of both muscle contractile function and biomechanical properties. Such properties are evaluated through precise measurements of changes in tissue outer diameter in response to intraluminal pressures in the presence or absence of muscle stimulants or relaxants. We believe that such a system will be useful in the elucidation of urethral pathophysiology and/or dysfunction as well as to assist in the development and evaluation of pharmacological, surgical, cellular, or gene therapies to address such conditions.

METHODS

Animals. Adult female, Sprague-Dawley rats (260–320 g, ~8–10 wk of age; Harlan) were used for tissue harvest. All animals were housed at the University of Pittsburgh under the supervision of the Department of Laboratory Animal Resources. The policies and procedures of the animal laboratory are in accordance with those detailed in the Guide for the Care and Use of Laboratory Animals published by the United States Department of Health and Human Services. Procedural protocols were approved by the Institutional Animal Care and Use Committee.

Urethra isolation and ex vivo testing apparatus. Intact urethras were isolated under halothane anesthesia (4%). The bladder and urethra were exposed via a lower midline incision, and a catheter (PE-50 tubing, OD = 0.965 mm) was inserted in the urethral lumen, extending the entire axial length and exiting from a hole placed in the bladder dome. The tissue was secured to the tubing with sutures at the midbladder and at the most distal portion of the urethra to maintain in vivo length after dissection. The ureters were ligated with sutures and...
served as an anatomic landmark by which the in vivo length was measured (typically 21–22 mm). This measured length was also used for positioning of the laser for regional measurements, as described below. The pubic bone was cut at a position lateral to the urethra and then separated and resected. The exposed urethra was gently removed from the ventral vaginal wall and the whole bladder-urethra unit was immediately placed in cold, oxygenated medium 199 (containing in g/l: 0.185 CaCl₂, 8.0 NaCl, 0.4 KCl, 0.097 MgSO₄ 0.06 K₂HPO₄, and 1.0 glucose; Sigma).

Before testing, the sutures securing the dissected urethra at in vivo length were cut, and the catheter was removed. The urethra was then secured with 4–0 sutures to stainless steel tubing and restored to in vivo length inside the experimental apparatus, which was a modification of an ex vivo vascular perfusion system previously described (14). Specifically, this system, used to accommodate a range of larger vascular tissue segments under continuous perfusion, was modified to accept the small-diameter urethras and to provide controlled fixed intraluminal pressures via an adjustable static fluid reservoir (Fig. 1A).

Zero pressure was set with the fluid reservoir level to the top of the bathing chamber; thus, the applied pressure reported is equal to the transmural pressure. The mounted urethra (Fig. 1, B and C) was enclosed within an 800-ml bathing chamber for maintenance of temperature and oxygenation via a 37°C circulation loop and connection to a physiological blood-gas mixture (21% O₂, 5% CO₂, 74% N₂) through the top of the chamber. Samples of bathing medium were periodically monitored for pH and oxygenation (pH 7.34–7.55, P O₂ 165–187 mmHg, and P CO₂ 20–30 mmHg; Radiometer ABL5). Medium 199 was used for both the bathing medium and to fill the hydrostatic pressure column, and tissues were allowed a minimum period of 30 min to equilibrate before testing. Air was removed from the urethral lumen by briefly unclamping the distal tubing and applying a small proximal pressure (typically 6–8 mmHg for visual fluid flow in the distal tubing), before returning to 0-mmHg pressure. A laser micrometer was positioned to measure urethral OD at chosen locations along the axial length. Proximal, mid, and distal regional measurements were performed by positioning the laser at axial positions 25, 50, and 75%, respectively, from the apex of the bladder (assumed to start 3 mm below the ureters), based on in vivo length. Both pressure and OD measurements were recorded simultaneously using LabView software.

**Normal tone vs. passive pressure-diameter responses.** Pressure-diameter (P-D) responses were examined for both normal and passive states of urethral tissue. Tissue in the normal state was not stimulated with agonists and thus exhibited only endogenous muscle tone. Immediately after collection of normal-state data, a passive state devoid of muscle tone was induced by addition of EDTA (3 mM final concentration) to the bath, allowing a 30-min equilibration period. These experimental conditions were confirmed to eliminate subsequent agonist-evoked muscle contraction in preliminary experiments; however, total calcium chelation in the passive state described here was not explicitly verified. Data were collected from tissue samples (n = 12) at all three urethral regions per sample in a random fashion. To minimize viscoelastic effects, a mechanical preconditioning protocol, consisting of 10 cycles of pressure steps from 0 to 8 mmHg, was performed before collection of P-D data. P-D responses became repeatable generally by the sixth to eighth inflation-deflation cycle, as observed through examination of resulting hysteresis loops. After preconditioning, the pressure was increased from 0 to 18 mmHg at 2-mmHg increments, and OD data were collected at 10 Hz over a 1-min period for each 2-mmHg step. The experimental pressure range was selected based on previous reported mean values of female rat bladder pressure (21), with an intentionally lower pressure range used in the preconditioning regimen to minimize potential damage to basal muscle elements. The OD data were then averaged at each incremental step to obtain discrete values for each value of applied pressure. This data analysis approach was taken since a pronounced creep effect after each pressurization step was not observed in either the normal or passive tissue states.

The P-D data were used to compute a bulk elastic tissue property in the normal and passive states, defined by tissue compliance (C) as

\[
C = \left( \frac{D_{\text{max}} - D_{\text{min}}}{D_{\text{min}}} \right) \times (P_{\text{max}} - P_{\text{min}})^{-1}
\]

For calculation of overall tissue compliance, D max and D min represent the OD at the maximum (P max, 18 mmHg) and minimum (P min, 0 mmHg) applied pressures, respectively. Because the overall compliance parameter does not allow detection of the differential responses seen at lower pressures, stepwise compliance using smaller pressure ranges was also examined. Stepwise compliance values were also determined from the same relationship, where the diameter values correspond to the maximum and minimum OD obtained from data analyzed at 4-mmHg incremental pressure ranges.

**Active contractile responses.** Stimulated urethral smooth and striated muscle contraction responses were also examined. For these experiments, the urethra was exposed to a fixed intraluminal pressure of 8 mmHg, which caused the tissue to be predilated and allowed for contraction. The deformation caused by this 8-mmHg applied pressure was measured at a single axial location (i.e., most proximal, mid, or distal) 30 min after pressurization and was subsequently used in determining the relative percentage change in OD measured after the addition of muscle-responsive agents, added consecutively: N°-nitro-L-arginine, a
nitric oxide synthase (NOS) inhibitor, 100 μM; phenylephrine (PE), an α₁-adrenergic receptor agonist, 40 μM; ACh, a nicotinic receptor agonist, 10 mM; and EDTA, a calcium chelator, 3 mM (n = 5–14 tissue samples/region; all chemicals obtained from Sigma). Dilution caused by EDTA was determined relative to the original 8-mmHg baseline. Additional studies were also performed with the following agents to examine their ability to block the chemically evoked contractile responses: sodium nitroprusside, a nitric oxide (NO) donor, 10 μM (n = 5); atrapine sulfate, a nonselective muscarinic antagonist, 1 μM (n = 12); hexamethonium, a neuronal nicotinic antagonist that acts as a ganglion-blocking agent, 100 μM (n = 12); and d-tubocurarine, a selective nicotinic antagonist, 100 μM (n = 2). All chemicals were obtained from Sigma, and all concentrations were based on previous in vitro urethral studies (5, 9, 16, 17, 25).

Each agent was added to the bathing medium circulation loop via an injection port and allowed 30 min to equilibrate before measurement of OD. The last 100 OD data points (collected at 1 Hz) of this equilibration period were averaged in the calculation of each relative response change. In addition, average OD change was calculated in response to ACh taken at the peak OD inflection point observed immediately after its addition to the bath. Preconditioning was not performed before the active tissue measurements because of concerns of compromising contractile function by repetitive stretching (8).

**RESULTS**

**Immunohistochemistry.** After P-D measurements, the tissue was removed from the testing apparatus and again secured to the catheter tubing with sutures at in vivo length. It was then placed in 2% paraformaldehyde solution for 24 h, followed by a 30% sucrose solution for an additional 1–2 h. Samples of the proximal, mid-, and distal segments, ~2 mm in axial length, were then cut with a razor, and the tubing was removed from the lumen of each segment. Each tissue segment was placed in TBS Tissue Freezing Medium (Triangle Biomedical Sciences), air was carefully removed from the lumen, and the segments were flash-frozen in liquid nitrogen. Frozen sections, 8 μm in thickness, were cut transverse to the longitudinal urethral axis using a cryostat (HM 505E; Microm) and mounted on microscope slides for immunohistochemical staining.

Striated and smooth muscle composition was determined from serial sections as follows. Sections were blocked with 5% goat serum and incubated with 1:250 dilutions of either mouse anti-skeletal slow myosin heavy chain (clone N0Q7.5.4D; Sigma) or a mixture of both anti-skeletal slow and fast (clone MY-32; Sigma) myosin heavy chain monoclonal antibodies for 2 h. For smooth muscle, sections were blocked with 5% goat serum and incubated with a 1:250 dilution of mouse anti-α-smooth muscle actin (Sigma) monoclonal antibody, also for 2 h. All sections were then washed with PBS, incubated with a Cy3-conjugated anti-mouse IgG antibody (1:250; Sigma) for 30 min, and washed again before immunofluorescence imaging. Negative control sections were treated in an identical fashion, with omission of the primary antibody.

For determination of total and fractional muscle area for each tissue region, digitized immunofluorescence images (Spot; Diagnostic Instruments) were acquired at ×40 magnification (E800; Nikon) and analyzed using Northern Eclipse software (Epix Imaging). Actual tissue cross-sectional area for each section was calculated as the difference in the areas enclosed by manually drawn borders of the luminal and abluminal surfaces. A threshold parameter was then applied to the image to distinguish the labeled-muscle immunofluorescence signal from the background. Smooth and striated muscle fractional composition for each region was determined by dividing the immunofluorescent-labeled muscle area by the tissue cross-sectional area. The percentage of each striated muscle fiber type was also determined individually from sections labeled with either slow or fast myosin heavy chain antibodies. For smooth muscle, areas occupied by large blood vessels were visually identified and excluded. For each region (proximal, mid, distal), values from three individual sections (one from each end and one from the middle of the tissue segment) were averaged to obtain a single volume fraction value representative of each tissue specimen and corresponding region. Individual specimen values were then averaged for all analyzed tissues (n = 4–6) to obtain the single reported value for each region.

**Statistical analysis.** For P-D data, comparison of overall and stepwise compliance values between the normal and passive states at each region were performed by Student’s t-test, or the equivalent nonparametric test when unequal variance was detected (Mann-Whitney Rank Sum test). One-way ANOVA with Student-Newman-Keuls’ post hoc pairwise comparisons, or an equivalent nonparametric test (ANOVA on ranks with Dunn’s post hoc analysis), was also used for multiple comparisons between all regions and tissue states for each calculated compliance.

For multiple comparisons between tissue regions after induced active contraction and relaxation responses, as well as for comparison of immunohistochemical results, one-way ANOVA or the equivalent nonparametric rank test was also used, as described above.

*P* values <0.05 are reported as significant, and data are expressed as means ± SE. All comparisons were performed using SigmaStat (v2.0; Jandel Scientific) statistical software.

**RESULTS**

**Normal and passive P-D responses.** Shown in Fig. 2 is the averaged P-D response of the urethral tissue over the entire applied transmural pressure range for both the normal and passive states. From this plot, it is apparent that the urethra responds to incremental increases in pressure in a nonhomogeneous fashion along the various regional axial positions. With endogenous smooth muscle tone intact, the tissue resists deformation at all three regions after the initial applied pressure.
steps (2–4 mmHg). In contrast, after elimination of muscle tone with calcium chelation (EDTA), immediate deformation is seen even at these low pressures, with a progressive stiffening as pressure is increased.

It is also apparent that the response in either state is highly nonlinear, an expected response from soft tissue. Comparison of bulk tissue properties through quantitative description of these P-D responses can be performed by comparing measures of compliance (Table 1). No difference in overall compliance over the entire applied pressure range was observed between the normal and passive states within the 0- to 4-mmHg pressure range (P = 0.131, 0.607, 0.564, for proximal, mid, and distal, respectively). However, multiple comparisons between all regions and tissue states did reveal a significant difference between proximal and distal passive responses. Stepwise compliance demonstrates a significant differential response between the normal and passive states within the 0- to 4-mmHg range (P < 0.01 for proximal and mid, P = 0.059 for distal; also P < 0.05 for passive proximal vs. passive distal, by multiple comparison). This relationship of increased compliance within the passive state is reversed within the 4- to 8-mmHg pressure range, as the basal muscle tone begins to lose its resistance to deformation and the passive tissue rapidly stiffens (P < 0.01 for proximal, mid, and distal comparisons). From 8 to 12 mmHg, both tissue states exhibit marked stiffening with similar values of compliance (P = 0.255, 0.084, 0.489 for proximal, mid, and distal, respectively).

Active muscle responses. Figure 3, A and B, provides examples of tissue contractions, reflected as decreases in OD, in response to smooth and striated muscle stimulation (n = 5–14 tissue samples/region). As shown in Fig. 3C, addition of an NOS inhibitor (Nω-nitro-L-arginine) resulted in a trend toward contraction at the proximal and mid regions (−19 ± 11% and −16 ± 7%, respectively) that was not apparent within the distal region (5 ± 10%; P = 0.212). After inhibition of NOS, stimulation with PE caused a similar degree of contraction within all three regions of the urethra (P = 0.732) that was continuous over the entire 30-min evaluation period. Subsequently, a biphasic response to ACh was observed, since both an immediate (<2 min) and a sustained (30 min) contraction was apparent for most tissues. A significantly greater degree of contraction was observed within the mid region at both time

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Table 1. Overall (0–18 mmHg) and stepwise compliance for tissue with (normal) or without (passive) basal smooth muscle tone

<table>
<thead>
<tr>
<th>Pressure Range, mmHg</th>
<th>Normal</th>
<th>Passive</th>
<th>Significance Detected (Normal vs. Passive)</th>
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<tbody>
<tr>
<td></td>
<td>Proximal</td>
<td>Mid</td>
<td>Distal</td>
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<tr>
<td>0–18</td>
<td>22±2</td>
<td>20±1</td>
<td>19±2</td>
</tr>
<tr>
<td>0–4</td>
<td>34±7</td>
<td>21±5</td>
<td>22±4</td>
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<td>4–8</td>
<td>37±6</td>
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<td>8–12</td>
<td>11±2</td>
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Values listed are means ± SE (×10⁻¹/mmHg). Compliance is representative of the fractional change in lumenal volume in response to a change in applied pressure.
points (immediate, −36 ± 4%; sustained, −17 ± 4%) compared with that observed in both the proximal (immediate, −8 ± 3%; sustained, −2 ± 5%) and distal (immediate, −3 ± 2%; sustained, 10 ± 3%) regions. The degree of tissue strain at the time of ACh addition, as determined through changes in OD relative to the 0 mmHg baseline, was not significantly different among the three regions (P = 0.332) and thus reduces the possibility of variations in contractile ability resulting from length-tension considerations. At the conclusion of this contraction protocol, addition of EDTA resulted in a rapid abolition of all contractile function with a shift in OD above the preestablished 8-mmHg baseline condition at all three regions, indicating a level of basal muscle tone in the unstimulated normal state (P = 0.129, comparison between regions).

**Smooth and striated muscle inhibition.** The ability of drugs to modulate smooth or striated muscle activity, as well as the biphasic ACh-induced contractile response, was examined. Addition of sodium nitroprusside eliminated 90 ± 8% of the basal muscle tone, as evidenced by only a slight residual relaxation after the subsequent addition of EDTA (Fig. 4B). In the presence of sodium nitroprusside, ACh-evoked immediate contractions were reduced and sustained contractions were almost completely eliminated within the mid region [−11 ± 4% immediate, and −3 ± 4% sustained, n = 5 (data not shown); compared with ACh alone, see Fig. 3C].

The nature of these reduced responses in the absence of smooth muscle activity and the elimination of the normal biphasic response to ACh were further investigated in separate studies within the mid region. In particular, the role of muscarinic or ganglionic neuronal nicotinic receptor activation in the ACh-induced contractions was examined through the use of specific inhibitors to these receptors. Prior addition of atropine (muscarinic) and hexamethonium (ganglionic; Fig. 4B) reduced the immediate ACh-evoked contractions (−36 ± 4%, ACh alone vs. −25 ± 4%, blocked; n = 12, P = 0.111) and almost completely eliminated the sustained contractions (−17 ± 4%, ACh alone vs. −1 ± 5%, blocked; P = 0.061). ACh-induced contractions were completely eliminated by blocking both nicotinic striated muscle and ganglionic receptors with d-tubocurarine, as well as muscarinic receptors with atropine, as shown in Fig. 4C (n = 2).

**Smooth and striated regional muscle content.** Immunohistochemical staining revealed a similar amount of smooth muscle within each region of the urethra (Fig. 5, left). However, when accounting for differences in tissue thickness, the smooth muscle component represented a significantly larger fractional percentage of the tissue within the distal region compared with both the mid and proximal regions (P < 0.05). A heterogeneous distribution of striated muscle was observed in terms of both total muscle and fractional composition within each region (Fig. 5, right). The largest amount (0.33 ± 0.02 mm²) was contained within the mid region, and the vast majority of this muscle was circumferentially oriented (as seen in Figs. 5 and 6). A significantly smaller amount (0.08 ± 0.01 mm²) of striated muscle was found within the distal region, compared with the mid region, in terms of both total muscle and fractional composition (P < 0.05). Distal region muscle also demonstrated a discontinuous and mixed arrangement of myofibers oriented in both the circumferential and longitudinal directions. Results of immunolabeling performed with mixtures of both slow and fast myosin heavy chain antibodies revealed that the striated myofibers within each of the three regions predominantly expressed the fast myosin heavy chain isoform (Fig. 6).

**DISCUSSION**

We have adapted an experimental technique previously employed for the ex vivo investigation of blood vessel mechanics and have demonstrated the feasibility of this approach for the study of the intact urethra. In validating this model, endogenous tissue tone and smooth muscle- and striated muscle-induced contractile responses, against applied intraluminal pressures, were recorded. Blocking studies demonstrate pharmacological control over specific activation or inactivation of these constituent muscle elements. Differential physiological responses were observed in the three axial regions examined, consistent with respective variations in local tissue structure.

Although the majority of investigations examining in vitro urethral function utilize strip or segmented ring preparations, use of whole-mounted preparations has been reported (3, 13, 20). In theory, whole preparations offer advantages over con-
ventional segmental methods because of the retention of natural tissue structure with minimal damage or disruption of muscle and structural fibers, as well as exposure to more physiological loading conditions. Previously described whole-mounted systems have been used to characterize overall urethral closure function through the measurement of parameters that are based on fluid flow resistance through the entire urethra (3, 13, 20). In contrast, strip or ring studies offer the ability to investigate specific axial regions of interest, which is critical for the study of regional functional responses as well as, for example, determining potential effects of therapies targeted to a particular segment of tissue. The system described here also permits regional measurements to be made, by adjusting the positioning of the laser micrometer, while retaining the whole tissue configuration. Furthermore, unlike conventional measurements of force, the currently described system permits contractile function of the tissue to be assessed, via diameter measurements, during application of a wide range of applied transmural pressure loads. Various mechanical parameters of the tissue can then be readily derived from these responses.

Fig. 5. Muscle composition within urethral axial regions (average numerical values, $n = 4–6$ preparations, are shown in top left corner of each image). Total smooth muscle area (left) remains relatively constant, whereas its fractional area varies between regions ($P < 0.05$, distal vs. proximal, mid). Striated muscle (right) is most prominent within the mid region, in terms of both total and fractional area ($P < 0.05$, mid vs. distal). All images taken at $\times 40$ magnification.

Fig. 6. Immunofluorescence labeling of serial sections within the mid region. The striated muscle layer is primarily composed of myofibers expressing fast myosin heavy chain (MHC), as demonstrated by the sharp reduction in the number of myofibers labeled by slow MHC antibodies (left; arrows highlight labeled fiber) compared with simultaneous labeling with both fast and slow MHC antibodies (right). Similar results were obtained within proximal and distal regions as well. Images taken at $\times 400$ magnification.
This ultimately permits a more thorough characterization of both regional biomechanical function and overall tissue properties, including relative contributions to these properties from individual tissue components.

Despite removal of the surrounding external support structure and neuronal input, our whole-mounted urethral preparation demonstrated a measurable basal muscular tone in the absence of applied stimulation. The intrinsic smooth and striated muscle tissue components also demonstrated distinct and measurable abilities to contract against applied intraluminal pressures when pharmacologically stimulated, either jointly or independently. Smooth muscle activity in this model was demonstrated by tissue responsiveness to NOS inhibitors and NO donors (\(\text{N}^6\)-nitro-L-arginine and sodium nitroprusside, respectively) and an α-adrenergic agonist (PE). Homogeneity in contractile function in response to PE stimulation corresponded to immunohistochemical observations of a constant and continuous smooth muscle component throughout the urethral axial length, also histologically confirmed through previous observations (18). In regard to the observed contractile effects of NOS inhibition (\(\text{N}^6\)-nitro-L-arginine), prior in vivo studies in the female rat have shown that urethral smooth muscle relaxation during reflex bladder contractions is at least partially mediated through the actions of NO (2). NOS has been localized within several different cell types of the lower urinary tract, including both uroepithelial cells lining the lumen of the urethra and neurons innervating the smooth muscle, and thus should be intrinsic to our whole specimen preparation (6, 7). Contractile responses to NOS blockade observed here (within proximal and mid, but not the distal region) would suggest a constitutive release of NO within these regions in the absence of neuronal input from the spinal cord. This is inconsistent with previous in vivo work demonstrating stable baseline urethral pressure in the presence or absence of NOS inhibitors (12). However, for many of our specimens, responses to NO inhibition were also not observed (5 of 11 for proximal, and 5 of 14 for mid) even though subsequent responses to both PE and ACh were detected. The source of this inconsistency, which contributed to a large degree of variability in the reported mean responses to NOS inhibition in this study, is currently unresolved. Possible variations imparted by denudation of the urothelium, via the catheter, were not evident from histological analysis (data not shown).

Striated muscle activity in this model was demonstrated by tissue responsiveness to a nicotinic receptor agonist (ACh) alone, and in the presence of both muscarinic (atropine) and ganglionic neuronal nicotinic (hexamethonium) antagonists. Results of the blocking experiments taken together with the observed biphasic response after ACh stimulation alone suggest a combined ACh-evoked smooth and striated muscle activation within the mid region. It follows that the immediate contractile response was the result of nicotinic receptor-mediated striated muscle contraction superimposed on a muscarinic-mediated smooth muscle contraction. After subsequent fatigue of the striated muscle and/or nicotinic receptor desensitization, only the sustained smooth muscle activity remained (visible at the 30-min time point). Such cholinergic-mediated excitatory mechanisms of both urethral striated and smooth muscle have also been demonstrated in previous in vivo studies in the rat (12). Heterogeneity in the immediate ACh responses corresponded with striated muscle area and fractional composition within the local axial regions of the tissue, as demonstrated histologically here. Previous histological observations in older (12–36 mo) female rats were comparable to the striated volume fractions and distribution reported here, although slow-twitch striated myofibers were found to predominate (18). Interestingly, however, the lack of sustained ACh-evoked contraction at both the proximal and distal regions, despite continuous smooth muscle content, may be indicative of regional variations in muscarinic receptor distribution among regions as well. Indeed, axial variations in contractility in the presence of muscarinic blockage have been recently reported in the male greyhound urethra (24).

Preliminary comparison of tissue properties with and without basal muscle tone was examined through P-D measurements. Total compliance over the entire pressure range used (0–18 mmHg), which is calculated from only the first and last P-D values, does not appear to be a useful parameter for such a comparison. Incremental compliance over smaller pressure ranges, such as the 4-mmHg increment used here, was more sensitive in evaluating the effects of this basal muscle tone and the range over which it exerts its influence. Although similarly obtained data do not currently exist with regard to the urethra, comparable studies in the area of vascular biomechanics have provided values for passive arterial (cerebral and carotid) compliance that compare favorably with the passive state urethral values reported here after exposure to similar pressures (~8–10 mmHg\(^{-1}\) up to 25 mmHg; see Ref. 11). Such comparisons provide us with some assurance that the values obtained here are within the range of those previously described for other passive tubular biological structures obtained using similar methodology. Future studies involving induced contractions of either smooth or striated components alone, or together, may allow investigation of the effect of activation of each of these urethral muscle components on such biomechanical properties. The relative importance of each component in this regard is a subject of continued debate within the urological community (22).

Slight modification to the experimental methodology described would enhance both the quality and amount of information that may be obtained from each experiment. As with any physiological muscle-based preparation, the viability of the tissue at the time of testing is paramount in obtaining representative and reproducible contractile responses. Adequate viability in our preparation is suggested through demonstration of reproducible urethromotor responses after urethral harvest and storage. Nonetheless, implementation of viability criteria before testing may prevent the introduction of additional variability in contraction and relaxation measurements. Other considerations for future studies, aside from those already mentioned, include expansion of the biomechanical parameters used for characterization of the urethra. This would presumably include an explicit assessment of urethral viscoelasticity as well as examination of some of the more well-established biomechanics parameters, such as incremental elastic modulus, that will allow for a more direct comparison with other cylindrical tissue types (i.e., vascular tissues) and serve to establish such baseline properties for the urethra. Calculation of such parameters mandates incorporation of tissue thickness and estimation of inner tissue radius at each applied load.
In summary, we have demonstrated the feasibility and usefulness of a modified vascular biomechanics system for the study of urethral function. The results presented here provide the foundation for future studies investigating regional urethral responses and further investigation of the biomechanical properties of both normal and diseased tissues. Such studies will be invaluable in both designing and evaluating therapeutic strategies to address underlying structural tissue changes resulting from acute injury or progression of disease states and their subsequent association with urethral dysfunction.

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