Effect of partial outlet obstruction on rabbit urinary bladder smooth muscle function

Xiaoling Su, Raymund Stein, Michaela C. Stanton, Stephen Zderic, and Robert S. Moreland

1Department of Pharmacology and Physiology, Drexel University College of Medicine, Philadelphia 19102; and 2Department of Urology, The Children's Hospital of Philadelphia, Philadelphia, Pennsylvania 19101

Submitted 29 July 2002; accepted in final form 29 November 2002

Su, Xiaoling, Raymund Stein, Michaela C. Stanton, Stephen Zderic, and Robert S. Moreland. Effect of partial outlet obstruction on rabbit urinary bladder smooth muscle function. Am J Physiol Renal Physiol 284: F644–F652, 2003; 10.1152/ajprenal.00274.2002.—Bladder outlet obstruction secondary to benign prostate hyperplasia is associated with many cellular changes. This study was designed to determine whether these changes involve the contractile apparatus. Bladder smooth muscles from rabbits subjected to partial outlet obstruction for 2 wk were mounted for isometric force, isotonic shortening velocity, and myosin light chain (MLC) phosphorylation levels. Muscle strips from obstructed bladders exhibited spontaneous phasic activity; muscle strips from control bladders did not. Muscle strips from obstructed bladders exhibited increased sensitivity and higher levels of stress in response to the cumulative addition of KCl or carbachol compared with control. During noncumulative addition of KCl or carbachol, no differences in sensitivity were noted. Muscle strips from obstructed bladders had elevated basal MLC phosphorylation levels and stimulation produced small increases in MLC phosphorylation compared with control. In KCl stimulation of muscle strips from obstructed bladders was 10-fold lower than control. Our results suggest that bladder outlet obstruction produces a muscle cell that develops higher levels of force but with greatly reduced cross bridge cycling rates.

benign prostatic hyperplasia; shortening velocity; myosin light chain phosphorylation; carbachol

THE PATHOLOGICAL PROGRESSION of untreated bladder outlet obstruction has been explained clinically by the concept of a detrusor muscle compensatory response. This concept assumes that in response to obstruction, the bladder smooth muscle hypertrophies to produce the elevated pressures necessary to maintain effective emptying. However, if the obstruction is left untreated, the bladder becomes dysfunctional, leading to a significant loss of contractile ability and an increase in postvoid residual volume. This is presumably due to an imbalance between the passive and active mechanical properties of the detrusor muscle and the magnitude of the resistance to flow. Removal of the obstruction before a state of severe dysfunction reverses the hypertrophic response, and normal function may be regained (7, 26).

In general, results from studies using animal models of bladder outlet obstruction report rapid and marked morphological and functional changes in the detrusor muscle, similar to those reported in human clinical studies (3, 6, 11, 17, 20, 23). The majority of these animal studies report a decrease in several parameters of detrusor contractility. This is also true using the model we employed in the present study, the acute partially obstructed rabbit urinary bladder model (15). In this acute animal model, partial obstruction results in 1) significant hypertrophy of the smooth muscle with a several-fold increase in bladder mass; 2) a decrease in the sensitivity to cholinergic stimulation of both the isolated whole bladder and isolated mucosal intact smooth muscle strips from the bladder body; 3) a decrease in absolute isometric force development using mucosal intact strips of bladder wall; and 4) an increase in postvoiding residual volume (14, 27, 29, 30). Our goal for the present study was to verify whether outlet obstruction decreases smooth muscle contractility, using a preparation containing primarily smooth muscle cells (devoid of both serosal and mucosal layers), and to ascertain the step(s) involved in the excitation process that may account for the obstruction induced changes.

More specifically, it is known that the time course of isometric force development of the normal intact rabbit bladder or isolated strips of bladder smooth muscle to agonist activation consists of two phases: an initial transient phase, in which force rises rapidly to a peak (phasic) then decaying slowly before attaining a steady level that is maintained for a prolonged period, and the tonic phase (24). In the hypertrophied bladder, both the phasic and tonic components of the isometric force response have been shown to be depressed, using mucosal intact bladder wall strips (15, 16, 27). In particular, it has been suggested that a reduced rate of force development and a significantly reduced ability to maintain force during the tonic phase occur in mucosal intact muscle strips from the obstructed bladder (15, 21). This present study was designed to verify these...
findings in a bladder strip preparation containing primarily smooth muscle cells and then examine the mechanism(s) potentially responsible for this altered mechanical performance.

MATERIALS AND METHODS

Animal model. Four-month-old male New Zealand White rabbits weighing 2.5–3.5 kg were used in this study. All animal studies were approved by the Children’s Hospital of Philadelphia Animal Care and Use Committee. Partial bladder-outlet obstruction was created as previously reported (21). Briefly, after the animal was anesthetized, an 8 French catheter was inserted into the bladder via the urethra, and the bladder neck was exposed through a small vertical extraperitoneal abdominal incision. The ureters and vas deferens were identified, and a 2-0 silk suture was placed below the bladder neck. To maximize standardization of the partial outlet obstruction, a second 8 French catheter was placed outside the urethra, and the silk suture was tied around both catheters. Both catheters were then removed. In the sham-operated group, the silk suture was placed around the catheterized urethra but not tied and then the catheter was removed. Data collected from sham-operated rabbits along with rabbits that did not undergo any surgical intervention were used as the control. The rabbits were housed in metabolic cages and monitored for voiding frequency and volume. Fourteen days after surgery to induce partial outlet obstruction, the animals were euthanized using IACUC-approved techniques, and the bladders were quickly removed.

Tissue preparation. The bladder neck, trigone, and base region were removed, leaving only middle detrusor body for experimentation. In all but one set of experiments, the mucosa and serosa were carefully removed under a dissecting microscope. In one set of experiments, the mucosal layer was retained. Muscle strips (~1.5 × 6 mm) were cut along the central axis of the bladder in the longitudinal orientation. At least four to eight strips were obtained from each bladder. The bladder strips were mounted in water-jacketed muscle chambers containing a MOPS-buffered physiological salt solution (18) at 37°C and aerated with 100% O2. The strips were equilibrated for at least 90 min. After an equilibration period, a partial length-tension curve was performed to determine the optimal length for active stress development (L0).

Tissues to be used for histological examination were fixed at L0, in buffered 10% formalin. The tissues were embedded in paraffin from which 5-μm longitudinal and transverse sections were cut and stained with hematoxylin-eosin and with Masson trichrome. Histological sections from the center of the embedded tissue strips were used for determination of proportion of smooth muscle to avoid any end effects from tissue clamps. Sections were magnified and projected onto a sheet of heavy paper. The total and smooth muscle specific areas were outlined, cut, and weighed. The ratio of the smooth muscle area to the total area was used to estimate the proportion of muscle in the tissue sections.

Measurement of contraction. Bladder strips used for isometric force recording were mounted between two plastic clips, one attached to a micrometer for length adjustment and the other to a Grass FT.03 force transducer and a Grass model 7D polygraph. Concentration-response curves were constructed by either the cumulative or noncumulative addition of KCl (equimolar substitution for NaCl) or carbachol. Each muscle strip was subjected to one of four protocols. Data obtained in these protocols are expressed as active stress (stress = force/cross-sectional area) or normalized as a percentage of the maximal response to 110 mM KCl. Cross-sectional area was determined using tissue length and weight as previously described (18).

Estimates of maximal velocity of shortening were performed by subjecting the bladder strips to a series of isotonic quick releases to afterloads ranging from 0.12 to 0.4 times the force at the instant of release as previously described for vascular smooth muscle (18). Strips were mounted on one end by a plastic clip attached to a micrometer for control of muscle length and on the other end by an aluminum foil tube connected to a Cambridge Technology 300H servo lever interfaced to Northstar Horizon computers. Isotonic shortening velocity at each afterload was estimated using the length change between 1 and 2 s after the release. A linearization of the hyperbolic force-velocity equation was used to estimate the maximal velocity of shortening during zero load.

Biochemical studies. For measurement of myosin light chain (MLC) phosphorylation levels, all strips were mounted, equilibrated, and then rapidly frozen at appropriate time points during a contractile event in a dry ice/acetone slurry containing 6% trichloroacetic acid and 10 mM DTT. The strips were then slowly thawed at room temperature. The tissues were rinsed in acetone and air-dried, and then dry weights were recorded. The acetone-dried tissues were homogenized in a solution containing 1% SDS, 10% glycerol, and 1 mM DTT using glass/glass homogenizers. The homogenates were clarified by centrifugation and then subjected to two-dimensional gel electrophoresis, followed by transfer to nitrocellulose membranes as previously described (19). Proteins were visualized using AuroDye forte colloidal gold protein stain (Amersham) and quantified using laser scanning densitometry (Molecular Dynamics). MLC phosphorylation levels were calculated as a percentage of the sum of the densitometric analysis of both the phosphorylated and unphosphorylated forms of the MLC.

Bladder strips were also processed for the determination of myosin-to-actin ratios with minor modifications of techniques previously reported (5). Briefly, aliquots of the homogenized tissues were subjected to SDS-PAGE. The separating gel was divided into two components; the bottom 5 cm of the gel contained 12% acrylamide, whereas the upper 7 cm contained 7.5% acrylamide. The stacking gel was the typical 4% acrylamide. The use of two distinct acrylamide concentrations provided better resolution for myosin heavy chain and actin on a single gel. Three different dilutions of each tissue homogenate were loaded onto the gels to ensure linearity of quantitation. After electrophoresis the gels were stained with Coomassie blue R-250. Band identity of myosin and actin was confirmed by immunoblots. Aliquots of the homogenized tissue samples that were used for determination of MLC phosphorylation were also used for determining total protein content by the Bradford method using bovine serum albumin as a standard.

Data analysis. All data are presented as means ± SE. Student’s t-test and ANOVA were used when it was appropriate. Values of P < 0.05 were taken as significant.

RESULTS

Partial outlet obstruction of the rabbit bladder produces several significant changes in both the structure and function of the organ. In terms of urinary output, Table 1 shows the data obtained from control and outlet-obstructed animals housed in metabolic cages. Animals subjected to outlet obstruction for 2 wk had significantly higher number of voids/day and significantly lower average volume/void. It is noteworthy that
Table 1. Functional and anatomic properties of control rabbits and rabbits subjected to partial bladder outlet obstruction

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Obstructed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Voiding frequency, no. of voids/24 h</td>
<td>5.5 ± 0.9 (12)</td>
<td>43.2 ± 4.2 (16)*</td>
</tr>
<tr>
<td>Maximum volume/void, ml</td>
<td>47.8 ± 1.0 (12)</td>
<td>12.3 ± 2.9 (16)*</td>
</tr>
<tr>
<td>Average volume/void, ml</td>
<td>27.8 ± 4.6 (12)</td>
<td>3.8 ± 0.6 (16)*</td>
</tr>
<tr>
<td>Total void volume/24 h, ml</td>
<td>114.1 ± 10.1 (11)</td>
<td>126.1 ± 9.8 (16)</td>
</tr>
<tr>
<td>Wet wt of bladder, g</td>
<td>3.28 ± 0.55 (10)</td>
<td>7.76 ± 0.23 (7)*</td>
</tr>
<tr>
<td>Time to maximal force, s</td>
<td>17.3 ± 1.2 (5)</td>
<td>55.9 ± 11.1 (7)*</td>
</tr>
<tr>
<td>Myosin heavy chain/actin</td>
<td>0.21 ± 0.04 (4)</td>
<td>0.21 ± 0.02 (4)</td>
</tr>
<tr>
<td>Mean strip wet wt, mg</td>
<td>1.20 ± 0.07 (12)</td>
<td>2.19 ± 0.15 (8)</td>
</tr>
<tr>
<td>Percent muscle mass</td>
<td>40.4 ± 2.5 (4)</td>
<td>45.2 ± 8.0 (4)</td>
</tr>
</tbody>
</table>

Values are means ± SE with no. of animals in parentheses. *P < 0.05.

total void volume/day is not different between the two animal groups. Bladder weights from obstructed animals were also significantly elevated compared with those from control animals, suggestive of obstruction-induced bladder hypertrophy.

It has been generally believed that bladder smooth muscles from animals subjected to partial outlet obstruction develop lower levels of maximal force (7, 14, 15). To confirm those results in our own laboratory, we measured the time course of maximal force development in bladder strips from control and obstructed animals. In these experiments, we used the typical bladder muscle strip in which the serosal layer but not the mucosal layer had been removed. The results, presented in Fig. 1, show that significantly less stress (force/cross-sectional area) is developed by the muscle strips from an obstructed animal (wet wt = 3.11 ± 0.35 mg; n = 6); compared with control (wet wt = 1.98 ± 0.23 mg; n = 6). This depression of stress development is noted in response to either agonist activation (10 μM carbachol; Fig. 1A) or membrane depolarization (stimulation by 110 mM KCl; Fig. 1B).

A decrease in stress can be due to either an increase in bladder wall nonmuscle mass or a decrease in muscle cell contractility. To determine whether muscle mass was altered in bladders from obstructed animals, we developed a tissue preparation devoid of both the serosal and mucosal layers and subjected the tissue containing predominantly a smooth muscle layer to histological examination as described in MATERIALS AND METHODS. Representative sections from a strip of control bladder smooth muscle and one from an animal subjected to partial outlet obstruction are presented in Fig. 2. Compared with the smooth muscle strip from a control animal, the tissue from the obstructed bladder shows gross changes in smooth muscle orientation. However, obstruction of the bladder did not alter the content of muscle area (40.4 ± 2.5% muscle in control tissues; 45.2 ± 8.0% muscle in tissue from obstructed bladders; n = 4 both groups). These qualitative changes in bladder wall cellular orientation demonstrate that partial outlet obstruction alters the cytoarchitecture of the tissue but not the percentage of muscle mass, at least in the smooth muscle layer. In terms of intracellular components, the data listed in Table 1 show that the ratio of myosin heavy chain to actin is also not altered by partial outlet obstruction.

The results obtained from Fig. 2 demonstrate that the percent muscle mass does not appear to be significantly different in tissues from the two animals groups. Therefore, a decrease in the contractility of the smooth muscle cells induced by partial outlet obstruction could account for the depression in force noted in Fig. 1. Cumulative and noncumulative concentration-response curves were constructed in response to KCl (Fig. 3, A and B) and carbachol (Fig. 4, A and B). In these and all subsequent experiments, we used the dissection technique that removed both the serosal and mucosal layers, resulting in a tissue strip with a higher percentage of smooth muscle cells. Strips of urinary bladder from partial outlet-obstructed animals produced more stress to the cumulative addition of either KCl or carbachol compared with strips from control
animals. Strips of bladder from obstructed animals were also more sensitive to either KCl (EC$_{50}$: 18 mM obstructed; 29.5 mM control) or carbachol (EC$_{50}$: 0.27 μM obstructed; 0.84 μM control) during the cumulative response experiments compared with tissue from control animals (Figs. 3A and 4A). There were no significant differences in the maximal levels of stress developed or sensitivity of response to KCl during the noncumulative response experiments using bladder strips from obstructed compared with control animals. There were also no significant differences in the sensitivity to carbachol during the noncumulative response between smooth muscle tissues from the two animal groups. The level of stress developed at the highest carbachol concentration (100 μM) was significantly less in smooth muscle from obstructed compared with control animals.

During the collection of data for the construction of the noncumulative concentration-response curves, one difference in the smooth muscles from the two animal sources was striking, that being the temporal profile of a single contractile event. Figure 5 shows the averaged results of several contractions of the bladder strips in response to 110 mM KCl. The rate of the initial phasic force development is significantly slower in bladder smooth muscle strips from control compared with muscle strips from partial outlet-obstructed animals (Table 1). Moreover, in contrast to the typical initial phasic contraction followed by the lower but suprabasal steady-state maintenance of force in bladder strips from control animals, bladders strips from the outlet-obstructed animals maintained peak forces longer and decayed significantly more slowly. It is also of interest to point out that the time course of a contraction in the mucosa-intact strip is prolonged compared with that in the strips dissected free of the mucosal layer. We believe this is most likely due to enhanced diffusional delays in the thicker mucosa-intact tissues. Figure 6 shows that the quasi-steady-state levels of force, as a percentage of peak force, are significantly higher in bladder strips from outlet-obstructed animals compared with those from control in response to several concentrations of carbachol. Thus the alteration of contractile profile is not stimulus dependent and instead is a fundamental change in the behavior of the muscle strip after partial outlet obstruction.

The primary step in the initiation of a smooth muscle contraction is the calcium- and calmodulin-dependent phosphorylation of the 20,000-Da MLC (9, 12). Thus it was important to determine whether the partial outlet obstruction-induced alterations in contraction were correlated to a change in MLC phosphorylation levels. We stimulated bladder strips from control and obstructed animals with 110 mM KCl, rapidly froze the tissues at various times during the contractile event, and processed the tissues for quantitation of MLC.
phosphorylation levels. The results of these experiments are shown in Fig. 7. Surprisingly, basal levels of MLC phosphorylation were significantly elevated in bladder strips from obstructed animals compared with those from control. Stimulated levels of MLC phosphorylation were not different from the two animal groups even though the temporal profile of force was significantly different. Of potential importance in terms of the elevated basal levels of MLC phosphorylation was the finding that all muscle strips from obstructed animals exhibited spontaneous phasic activity, whereas this was noted in <10% of muscle strips from control animals.

Several groups have shown that after partial bladder outlet obstruction, the primary isoform of myosin in the smooth muscle cells changes from SM-B to SM-A (2, 10, 28). The SM-A isoform of myosin is characterized by a slower actin-activated myosin ATPase activity. We were therefore interested in determining

Fig. 3. KCl concentration-response curves using rabbit bladder smooth muscle. A: strips of bladder smooth muscle dissected free of both serosal and mucosal layers were subjected to the cumulative addition of KCl from 4.7 to 110 mM. Smooth muscle strips from control bladders (●) produced lower levels of stress and were less sensitive in response to the addition KCl compared with smooth muscle strips from partially obstructed bladders (○). Maximal levels of stress were developed at 30 mM in strips from partially obstructed bladders and 80 mM in strips from control bladders. The calculated EC50 values were 18 mM for strips from partially obstructed bladders and 29.5 mM for control. Values are means ± SE; n = 5–8.

B: strips of bladder smooth muscle from control (●) and partial bladder-obstructed animals (○) were subjected to the noncumulative addition of KCl. There were no differences in either maximal levels of stress attained or in the sensitivity to KCl between strips from control compared with those from partial bladder-obstructed animals. The calculated EC50 values were 54.1 mM for strips from control bladders and 37.7 mM for those from obstructed bladders. Values are means ± SE; n = 5–8.

Fig. 4. Carbachol concentration-response curves using rabbit bladder smooth muscle. A: strips of bladder smooth muscle dissected free of both the serosal and mucosal layers were subjected to the cumulative addition of carbachol from 0.01 to 100 μM. Smooth muscle strips from control bladders (●) produced lower levels of stress and were less sensitive in response to the addition carbachol compared with smooth muscle strips from partial outlet-obstructed animals (○). Maximal levels of stress were developed at 3 μM in strips from partial outlet-obstructed animals and 100 μM in strips from control animals. The calculated EC50 values were 0.27 μM for strips from partial outlet-obstructed animals and 0.84 μM for control. Values are means ± SE; n = 5–8.

B: strips of bladder smooth muscle from control (●) and partial outlet-obstructed animals (○) were subjected to the noncumulative addition of carbachol. There was no difference in the sensitivity to carbachol between strips from control compared with those from partially obstructed bladders. There was a significant difference in the maximal stress developed only at the highest carbachol concentration. The calculated EC50 values 0.57 μM for strips from control and 0.42 μM for those from obstructed animals. Values shown are means ± SE; n = 5–8.

AJP Renal Physiol • VOL 284 • APRIL 2003 • www.ajprenal.org
whether the shortening velocities of bladder strips were similarly altered after partial outlet obstruction. Bladder strips from control and outlet-obstructed animals were stimulated with 110 mM KCl and then subjected to several isotonic releases at 5, 15, and 30 s of contraction. The maximal velocities of shortening ($V_o$) were estimated as described in MATERIALS AND METHODS and are shown in Fig. 8. $V_o$ of the muscle strips from control animals declines with time of stimulation, as has been shown in most smooth muscles examined (18). $V_o$ of the muscle strips from obstructed animals was more than an order of magnitude lower than that from control. We also performed a limited number of force redevelopment experiments using carbachol as the stimulus. Force redevelopment during carbachol stimulation was consistently slower in strips from outlet-obstructed animals compared with strips from control animals (data not shown). These results are consistent with the biochemical studies demonstrating a change in SM-A, the myosin isoform with a lower actin-activated myosin ATPase activity (13, 22).

DISCUSSION

The results presented in this study clearly show that smooth muscle tissue of the rabbit urinary bladder
undergoes significant functional alterations in response to partial outlet obstruction. In our opinion, the most striking of these alterations is the significant increase in spontaneous phasic activity and the maintenance of high levels of force after stimulation in bladder smooth muscle from partial outlet-obstructed animals.

Also of potential interest is the finding that bladder wall strips from animals subjected to partial outlet obstruction dissected free of only the serosal layer developed less stress compared with strips from control animals. This is in contrast to the finding that bladder wall strips from both control and outlet-obstructed animals dissected free of both serosal and mucosal layers developed similar levels of stress. Because stress is calculated as force/cross-sectional area, it is possible that the hypertrophied mucosal layer in the bladders from partial outlet-obstructed animals increased cross-sectional area and thus decreased stress. However, levels of actual force were also lower in those tissues from obstructed animals containing a mucosal layer. A more plausible explanation is that an altered matrix within the mucosal layer impedes contractile activity, resulting in lower levels of force development. Due to this possibility and because we were interested in examining the smooth muscle cells as directly as possible, all subsequent studies were performed using a mucosal and serosal-free preparation. It is important to note that it is well documented that partial bladder outlet obstruction induces numerous changes in contractile protein isoform, changes in expression levels of contractile regulatory proteins, and changes in calcium handling in the bladder smooth muscle cell (2, 7, 10, 21, 26, 29). Thus based on the finding that the stress generation of the muscles from the two sources is similar, we interpret this to suggest that the numerous and widespread changes may be compensatory in nature and important in maintaining bladder function in the face of an obstruction. Based on the categories of partial outlet obstruction as suggested by Levin et al. (7), we would classify our results as applying to the late compensated or early decompensated state. This compensated state is one in which contractile function and bladder weight have stabilized before going into the failing or severely decompensated state. However, bladder function was compromised, as shown by the data in Table 1, hence the placement in the early decompensated state.

Bladder smooth muscle strips from partial outlet-obstructed animals showed no significant differences in either the sensitivity or magnitude of contraction in response to the noncumulative addition of KCl and only a small difference in magnitude with the noncumulative addition of carbachol. In contrast, smooth muscle from the obstructed animals showed enhanced sensitivity and higher levels of force to both KCl and carbachol during the cumulative additions. We believe the results presented in Figs. 3A and 4A demonstrate that partial outlet obstruction produces a significant loss of the mechanism(s) responsible for desensitization of smooth muscle contraction. We also propose that bladder smooth muscle from outlet-obstructed rabbits may be an excellent model for the study of the mechanism(s) underlying receptor and contractile desensitization.

McConnell and colleagues (4) have shown that stimulation-induced maximal levels of MLC phosphorylation are similar in bladder smooth muscle strips from partial outlet-obstructed and control animals. Our results support these earlier findings. In addition, we provide results showing that basal values of MLC phosphorylation are elevated in bladder smooth muscle from obstructed animals compared with those from control. The impact of an elevated basal value of MLC phosphorylation with no change in stimulation-induced values is a decrease in the MLC phosphorylation dependence of contraction. The elevated basal values of MLC phosphorylation may also provide insight into the significant increase in spontaneous phasic activity in smooth muscle from obstructed animals compared with control. MLC phosphorylation and contraction are both calcium-dependent events. It is reasonable to assume that the increase in basal values of MLC phosphorylation and spontaneous activity is related and that both may be due to an increased calcium leak across the smooth muscle plasma membrane. This would be consistent with the well-described changes that occur in vascular smooth muscle during most forms of hypertension. Tonic vascular tissue from hypertensive animals has been shown to produce spontaneous phasic contractions and that this is the result of an increase in calcium influx from the extracellular space (8).

Biochemical studies have shown that, after partial outlet obstruction, the smooth muscle cells undergo a
change in the predominant isoform of myosin (2). Wang et al. (28) have presented evidence demonstrating that bladder smooth muscle from control animals contains predominately the SM-A isoform of myosin, whereas bladder smooth muscle from obstructed animals contains predominately the slower SM-B isoform of myosin. It has long been accepted that maximal velocity of shortening measurements provides an excellent estimate of myosin ATPase activity (1). This information provided the rationale for performing the mechanical characterization of the intact bladder smooth muscle from the two animal groups. Our results on maximal isometric shortening velocity in intact tissue are consistent with the biochemical evidence that smooth muscle from the bladder of partial outlet-obstructed animals contains a slower isoform of myosin compared with that from control rabbits.

Typically, stimulation of bladder smooth muscle produces an initial phasic contraction followed by a significantly lower sustained tonic phase. Bladder smooth muscles from animals subjected to partial outlet obstruction express a significantly altered contractile profile (21, 29). The phasic portion of a contraction of smooth muscle from the outlet-obstructed animals is prolonged to the point of approaching a tonic contraction. As shown in Figs. 5 and 6, quasi-steady-state levels of force are close to that developed at the peak of the phasic contraction. Our present studies do not address the mechanism(s) responsible for this high level of maintained force after partial outlet obstruction. However, depending on how one looks at the problem, it is possible to suggest plausible speculations. The transient nature of a contraction of bladder smooth muscle from control animals may be due to an active relaxation process or as a result of a rapid transient increase in activator calcium. The simplest explanation for the slow decrease in force in bladder strips from obstructed animals is higher intracellular calcium levels at any time during the contractile event. If the transient increase in calcium is prolonged, then one would expect a prolonged transient contraction. This possibility is supported by the higher basal values of MLC phosphorylation and the increase in spontaneous contraction. This possibility is not supported by the lack of change in either peak values or temporal profile of stimulation-induced increases in MLC phosphorylation. It is also interesting that the time course of a contraction in the mucosal intact strip is prolonged compared with that in the strips dissected free of the mucosal layer. We believe this is most likely due to enhanced diffusional delays in the thicker mucosal intact tissues.

A more complex explanation for the maintenance of force in muscle from obstructed animals could be a decrease in an active relaxation process. If one assumes that the transient nature of the bladder muscle contraction is the result of active relaxation, then any loss in this mechanism would produce a more tonic-like contraction. If this were the case, then the maintained force in muscles from obstructed animals could be due to either an alteration in the mechanism(s) responsible for active relaxation or a change in the tissue that opposes relaxation. This present study does not address these possibilities. However, we have presented preliminary information suggesting that the PKC-dependent pathway for contraction of bladder smooth muscle is either absent or constitutively active in tissues from obstructed bladders (25). If constitutively active, then this may explain the maintained contraction. Alternatively, it is well accepted that after partial outlet obstruction, the bladder matrix significantly increases in content. Any increased stiffness due to matrix materials once contracted would oppose an active muscle relaxation. What is clear however, is that temporal profile of a smooth muscle contraction from the partial outlet-obstructed animals is significantly different from that of control muscles.

Significant changes in smooth muscle have been shown to occur in most if not all pathophysiological states involving hollow organs. Our present study confirms and expands on the previous studies, showing that partial outlet obstruction secondary to benign prostate hyperplasia alters the functional status of the bladder smooth muscle. These changes include a prolonged contractile response to normal stimulation, a change in the mechanism of contractile desensitization, an alteration in basal MLC phosphorylation levels, and a decrease in the cross bridge cycling rate. It is now important to direct attention to determine how these alterations impact on micturition, whether continued obstruction induces a severely decompensated state, and whether removal of the obstruction reverses the change.

This work was supported in part by funds from National Institute of Diabetes and Digestive and Kidney Diseases O’Brien Center Grants DK-52620 (University of Pennsylvania Medical Center) and DK-57252 (R. S. Moreland).

REFERENCES


