Partial bladder outlet obstruction alters Ca\(^{2+}\) sensitivity of force, but not of MLC phosphorylation, in bladder smooth muscle

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Stanton, Michaela C., Michele Clement, Edward J. Macarak, Stephen A. Zderic, and Robert S. Moreland. Partial bladder outlet obstruction alters Ca\(^{2+}\) sensitivity of force, but not of MLC phosphorylation, in bladder smooth muscle. Am J Physiol Renal Physiol 285: F703–F710, 2003.—Partial bladder outlet obstruction in the rabbit produces changes in bladder function similar to those seen clinically in patients with obstructive uropathies. Whole organ function is significantly altered, as are the smooth muscle cells inside the bladder wall. This study was designed to determine whether outlet obstruction alters smooth muscle function at the level of contractile filaments. Rabbit bladders were partially obstructed for 2 wk. Triton X-100 was used to provide a detergent-skinned bladder smooth muscle preparation that would allow control of the intracellular environment while the ability to shorten and develop force is maintained. Ca\(^{2+}\)-force and Ca\(^{2+}\)-myosin light chain (MLC) phosphorylation relations and maximal velocity of shortening were determined. The Ca\(^{2+}\) sensitivity of force was significantly lower in tissues from animals subjected to outlet obstruction compared with tissues from control animals. In contrast, no difference was noted in the Ca\(^{2+}\) sensitivity of MLC phosphorylation. Maximal levels of stress and MLC phosphorylation were similar in both animal groups. Maximal velocity of shortening was significantly slower in tissues from animals subjected to outlet obstruction compared with tissues from control animals. Ultrastructurally, detergent skinning had little effect on structural integrity. Moreover, tissues from obstructed animals showed an increase in the number of sarcolemmal attachment plaque structures. We suggest that partial bladder outlet obstruction produces deleterious (e.g., decrease in Ca\(^{2+}\) sensitivity of force) and compensatory (e.g., increase in membrane attachment plaques) changes in bladder smooth muscle cells.

maximal velocity of shortening; Triton X-100 detergent-skinned fibers; attachment plaques; electron micrographs

ANIMAL MODELS ARE USEFUL TOOLS to study the effects of partial bladder outlet obstruction on muscle physiology. Some of the changes in muscle function that occur in such models appear to mimic some of the changes seen in certain clinical uropathies, such as prostatic hyperplasia (15, 35). The rabbit model has been used extensively, and information derived from these studies has provided important advances in our knowledge of how outlet obstruction impacts bladder function (16, 36). Bladders subjected to outlet obstruction undergo specific alterations in expression of contractile, regulatory, and structural proteins, leading to a shift in contractile properties from a phasic to a more tonic-like contraction (1, 3, 5). In addition, we have recently shown that bladder strips devoid of mucosal and serosal layers develop similar levels of force (31). The similarity in force development in bladder smooth muscle is not due to a change in percent muscle mass in the hypertrophied compared with the control bladders. Therefore, one plausible interpretation is that outlet obstruction-induced changes in intracellular components of the smooth muscle cell are compensatory at the contractile protein level to maintain normal levels of force in the face of the increased resistance.

Contraction of smooth muscle, including bladder muscle cells, is initiated by stimulation-induced increases in cytosolic free Ca\(^{2+}\) concentration. The cytosolic Ca\(^{2+}\) binds to calmodulin, and the Ca\(^{2+}\)-calmodulin complex activates the enzyme myosin light chain (MLC) kinase. Active MLC kinase catalyzes phosphorylation of the 20-kDa MLC, which activates the myosin molecule and allows it to interact with actin, with the resultant increase in cross-bridge cycling and force development (for review see Ref. 10). Dephosphorylation of the MLC by a MLC phosphatase initiates relaxation of the muscle cell. In addition to this primary pathway for excitation-contraction coupling, there are also several modulatory pathways that both increase and decrease the sensitivity of the contractile filaments to Ca\(^{2+}\) (26) as well as the latch state, in which high force can be maintained in the absence of proportional

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levels of MLC phosphorylation (21). However, it is clear that Ca$^{2+}$-dependent MLC phosphorylation is an important initiating step in smooth muscle contraction. Thus an alteration in the Ca$^{2+}$-MLC phosphorylation-force relation would be one likely mechanism that could account for the higher levels of force necessary to overcome increased resistance and to maintain force for longer periods of time, as in the switch from a phasic to a tonic contractile profile.

Therefore, the goal of this study was to determine whether partial bladder outlet obstruction has direct effects on the contractile apparatus of the detrusor smooth muscle cells. To approach this problem, we employed the Triton X-100 detergent-skinned preparation, which allows precise control of the muscle cell intracellular environment while maintaining its ability to develop force and shorten. If partial bladder outlet obstruction alters either Ca$^{2+}$-dependent force or MLC phosphorylation or the MLC dephosphorylation step, then this preparation will allow direct assessment of the change. On the other hand, if no alteration in any parameter of contraction is noted, then this will suggest that obstruction alters one of the modulatory or regulatory pathways not present in the Triton X-100 detergent-skinned preparation. Thus, regardless of the actual results, the information gained using this preparation will be important. We also examined the detergent-skinned preparation at the ultrastructural level to determine the effect of partial bladder outlet obstruction or detergent skinning on the structural integrity of the tissue.

**MATERIALS AND METHODS**

**Surgical techniques.** Mature male New Zealand White rabbits weighing 1.8–2.2 kg were used in this study. All animal studies were approved by the Institutional Animal Care and Use Committees of Drexel University College of Medicine and The Children’s Hospital of Philadelphia. Partial bladder outlet obstruction was created using an extraperitoneal approach with minimal bladder neck dissection. Catheters (8-Fr) were placed inside and outside the surgical exposure urethra, and 4.0 silk was used to ligate the urethra. Both catheters were then removed. Sham surgeries were identical, except the ligature was cut and removed before the wound was closed. Nonoperated rabbits served as an additional control. All bladders were harvested after 2 wk of obstruction and immediately placed in ice-cold physiological salt solution containing (in mM) 140 NaCl, 4.7 KCl, 1.2 MgSO$\text{\textsubscript{4}}$, 1.6 CaCl$\text{\textsubscript{2}}$, 1.2 Na$\text{\textsubscript{2}}$HPO$\text{\textsubscript{4}}$, 20.3 (N-morpholino)propanesulfonic acid, 5.0 d-glucose, and 0.02 Na$_2$EDTA.

**Tissue preparation.** The predominantly smooth muscle layer of the bladder wall was dissected free of both serosal and mucosal layers, and strips were prepared for measurement of isometric force, isometric shortening velocity, and quantification of MLC phosphorylation levels. Longitudinal strips measuring $-1.5 \times 6$ mm were cut from the middle portion of the detrusor body.

Isometric force was measured using strips mounted between two plastic clips in water-jacketed muscle chambers aerated with 100% O$_2$. One clip was attached to a micrometer for length control and the other to a force transducer (model FT.03, Grass Instrument) and a polygraph (model 7D, Grass Instrument). The strips were allowed to equilibrate for 90 min at 37°C and stretched to a length that approximates the optimal length for maximal active contraction (31).

All muscle strips were detergent skinned using a 0.5% Triton X-100 solution. The equilibrated intact strips were exposed to Ca$^{2+}$-free physiological salt solution for 30 min and then placed in a solution containing 5 mM EGTA, 20 mM imidazole, 50 mM potassium acetate, 1 mM dithiothreitol (DTT), 150 mM sucrose, and 0.5% Triton X-100 for 60 min. The strips were then exposed for 10 min to high-EGTA (5.0 mM) and then low-EGTA (0.2 mM) relaxing solutions, respectively, containing 20 mM imidazole (pH 6.8), 50 mM potassium acetate, 6 mM MgCl$_2$, 6 mM ATP, and 1 mM DTT. All experiments using detergent-skinned strips were performed at room temperature, pH 6.8, and at an ionic strength of 120 mM. Ca$^{2+}$-contracting solutions contained 1 mM free Mg$^{2+}$, 4 mM MgATP, 1 mM DTT, 5 mM EGTA, 20 mM imidazole (pH 6.8), sufficient potassium acetate to maintain ionic strength constant, and sufficient CaCl$_2$ to achieve the appropriate free Ca$^{2+}$ concentration. The amounts of total compound added to achieve the appropriate free concentration were calculated using a computer program to solve the simultaneous multiequilibrium equations, as previously described (19).

**Mechanical measurements.** Isotonic shortening velocity measurements were performed using strips mounted on one end by a plastic clip attached to a micrometer for control of muscle length and on the other end to an aluminum foil tube connected to a servo-lever (model 300H, Cambridge Technology) interfaced to a Linux operating system-based personal computer. The detergent-skinned strips were exposed to solutions containing, in addition to appropriate compounds to reflect intracellular conditions, 1.0, 3.0, and 20.0 µM Ca$^{2+}$. After stable force was achieved at each Ca$^{2+}$ concentration, the strips were subjected to isotonic quick releases to afterloads ranging from 5–20% of the initial force at the time of release. The change in length at each afterload was fit by a double-exponential equation, and a tangent to the fit at 100 ms after release was taken as the isotonic shortening velocity at that afterload. Isotonic shortening velocities at several afterloads were used to extrapolate velocity at zero load for calculation of maximal shortening velocity at each Ca$^{2+}$ concentration.

**MLC phosphorylation.** For determination of MLC phosphorylation levels, detergent-skinned strips were rapidly frozen by immersion in a dry ice-acetone slurry containing 6% (wt/vol) trichloroacetic acid and 10 mM DTT. The muscle strips were allowed to slowly thaw to room temperature, rinsed for 30 min in acetone, and homogenized on ice. The homogenization buffer contained 1.0% sodium dodecyl sulfate, 10% glycerol, and 20 mM DTT. Homogenized strips were subjected to two-dimensional electrophoresis followed by transfer to nitrocellulose membranes, as previously described (20). Proteins were visualized using colloidal gold stain (Amersham Pharmacia Biotech). MLC was quantified by scanning densitometry using a scanning densitometer (model GS 800, Bio-Rad). Values are reported as moles of P$_i$ per mole of MLC and were calculated by taking the volume of the densitometric spot representing monophosphorylated MLC and stretched to a length that approximates the optimal length for maximal active contraction (31). Values are reported as moles of P$_i$ per mole of MLC and were calculated by taking the volume of the densitometric spot representing monophosphorylated MLC and nonphosphorylated MLC.

**Electron microscopy.** Bladder wall dissected free of both serosal and mucosal layers was detergent skinned as described above. The skinned strips were then fixed in place at physiological lengths by immersion in a high-EGTA relaxing solution containing 1.5% (vol/vol) glutaraldehyde for 2 h. The tissues were rinsed in high-EGTA relaxing solution, dehydrated in ethanol, and then embedded in LR White resin.
Thin sections were collected on grids, and then the sections were contrasted with 3% uranyl acetate and examined in a transmission electron microscope (model 100CX, JEOL).

Data analysis. Values are means ± SE. Student’s t-test was used for unpaired data. P < 0.05 was taken as significant.

RESULTS

The data in Table 1 show the functional measurements of the rabbit bladder in control and sham-operated animals combined and in animals subjected to partial outlet obstruction for 2 wk. The animals subjected to partial bladder outlet obstruction have significantly higher voiding frequencies and lower urinary volumes per void. The bladders from the obstructed group also have significantly greater wet weights.

The sensitivity of intact bladder smooth muscle to the noncumulative addition of carbachol or KCl is not different in bladder smooth muscle from control animals and animals subjected to partial bladder outlet obstruction (29). The intact tissue, however, does not allow a direct examination of the contractile apparatus. We therefore performed studies using the Triton X-100 detergent-skinned preparation. Triton X-100 detergent-skinned strips of bladder smooth muscle were subjected to the noncumulative addition of various free Ca^{2+} concentrations. The results of these experiments are shown in Fig. 1. The addition of Ca^{2+} to detergent-skinned strips produced a concentration-dependent increase in stress (force/cross-sectional area) in smooth muscle from both animals groups. However, the Ca^{2+} sensitivity of stress development is significantly less in smooth muscle from animals subjected to partial bladder outlet obstruction than in smooth muscle from control animals: EC_{50} = 1.2 ± 0.2 μM (n = 6) vs. 6.3 ± 0.4 μM (n = 7). The maximal stress generated by the Triton X-100 detergent-skinned fibers was significantly greater than that developed by the intact preparations: intact control 7.9 ± 0.7 × 10^{4} vs. detergent-skinned control 3.1 ± 0.5 × 10^{5} N/m^{2} (qualitatively similar results were obtained in tissues from obstructed animals).

MLC phosphorylation is the predominant step in the initiation of a smooth muscle contraction. To fully understand the effect of partial bladder outlet obstruction on the Ca^{2+}-dependent regulation of contraction, one needs to know the Ca^{2+} dependence of MLC phosphorylation. The tissues in which Ca^{2+}-dependent stress was obtained for Fig. 1 were frozen after a stable force recording was attained, usually within 10 min of contraction, for quantitation of MLC phosphorylation levels. The resultant data are shown in Fig. 2. Similar to the results of maximal stress development, there were no differences in the maximal levels of MLC phosphorylation attained in bladder smooth muscle strips between control animals compared with those subjected to partial bladder outlet obstruction. In contrast to the results shown in Fig. 1, however, there were no trends, let alone significant differences, in the Ca^{2+} dependence of MLC phosphorylation between the muscle strips from the two animal groups.

Several biochemical studies have clearly shown that partial bladder outlet obstruction changes the isoform of myosin from the faster SM-B isoform to the slower SM-A isoform (2, 9), which translates to a decrease in the noncumulative addition of various free Ca^{2+} concentrations (|Ca^{2+}|) and allowed to reach steady-state levels of force. Force was measured, as was tissue length and then weight, for determination of cross-sectional area. All values are presented as stress (force/cross-sectional area). Detergent-skinned tissues from obstructed animals exhibited a significant decrease in Ca^{2+} sensitivity of force compared with tissues from control animals. There were no differences in the maximal value of stress attained. Values are means ± SE for ≥6 determinations. *Significantly different from control (P < 0.05).

Table 1. Functional measurements of rabbit bladder in control and sham animals and animals subjected to partial outlet obstruction

<table>
<thead>
<tr>
<th></th>
<th>Control (n = 6)</th>
<th>Obstructed (n = 8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Voiding frequency/24 h</td>
<td>8.1 ± 0.8</td>
<td>50.6 ± 14.0*</td>
</tr>
<tr>
<td>Average volume per void, ml</td>
<td>18.1 ± 2.5</td>
<td>3.5 ± 0.8*</td>
</tr>
<tr>
<td>Bladder wet wt, g</td>
<td>2.48 ± 0.28</td>
<td>11.9 ± 1.71*</td>
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Values are means ± SE. *Statistically different from control (P < 0.05).
cessed them for examination at the level of the electron microscope. Despite detergent treatment, structural preservation is quite good. In normal (Fig. 4A) and obstructed tissue (Fig. 4B), nuclear and cytoplasmic structures are evident. The sarcolemma and intracellular compartments are evident in Fig. 4. Note the close apposition of adjacent smooth muscle cells, the structural detail of the sarcolemma, dense bodies within the cytoplasm, and cytoskeletal filaments.

Of particular interest are the junctional structures that exist between smooth muscle cells consisting of electron-dense plaque-like structures immediately be-

Fig. 2. Myosin light chain (MLC) phosphorylation in Triton X-100 detergent-skinned tissues of bladder smooth muscle from control animals (○) and animals subjected to partial bladder outlet obstruction (●). Detergent-skinned tissues from control and obstructed animals were contracted in response to various free Ca\(^{2+}\) concentrations and allowed to reach steady-state levels of force. Tissues were then frozen and processed for quantitation of MLC phosphorylation levels. There were no significant differences in Ca\(^{2+}\) sensitivity of MLC phosphorylation or maximal values of MLC phosphorylation between tissues from control and obstructed animals. Values are means ± SE for ≥6 determinations.

Fig. 3. Maximal velocity of shortening in Triton X-100 detergent-skinned preparation of bladder smooth muscle. Detergent-skinned strips of muscle from control animals (solid bars) and animals subjected to partial bladder outlet obstruction (open bars) were mounted for isotonic force recording. Strips were contracted to 1, 3, or 20 μM Ca\(^{2+}\) and, when steady-state force was attained, subjected to several quick releases to various afterloads. Maximal velocity of shortening was calculated using isotonic shortening velocities at each afterload taken at 100 ms after the quick release and extrapolating to zero load. Maximal velocity of shortening is significantly lower in detergent-skinned strips from outlet-obstructed animals compared with those from the control animals at all Ca\(^{2+}\) concentrations. Values are means ± SE for ≥6 determinations. *Statistically different from control, \(P < 0.05\).

Fig. 4. Electron micrograph of Triton X-100 detergent-skinned bladder smooth muscle from control animals and animals subjected to outlet obstruction. A: control bladder tissue. Black arrows indicate the presence of plaque-like dense bodies at the level of the plasma membrane, the structure of which has been obviously disrupted by Triton X-100 treatment. White arrows denote the presence of cytoplasmic dense bodies. B: tissue from outlet-obstructed animals. Note extensive dense bodies associated with disrupted plasma membranes in obstructed bladder tissue (black arrows) and duplicated basement membranes extracellularly (white arrow).
neath the sarcolemma. These plaque-like structures are sometimes paired, being present in both adjacent smooth muscle cells whereas in other instances, they exist as single structures present in only one cell. Tissue from control animals (Fig. 4A) and tissue from obstructed animals (Fig. 4B) contain these plaque-like structures. Of interest is the apparent increase in the plaque-like structures in tissue from obstructed animals compared with control animals. These regions are where cytoskeletal proteins come into close apposition with the sarcolemma, likely providing structural rigidity to these sites, where tensional forces are transferred from the smooth muscle cell to both the adjacent smooth muscle cells and the extracellular matrix. This is shown most clearly in Fig. 5 (obstructed animal), where an interstitial fibroblast nucleus can be seen in the extracellular matrix between the two smooth muscle cells. Note also the very extensive single plaque-like structures directly adjacent to the extracellular matrix.

DISCUSSION

It is well documented that partial bladder outlet obstruction produces numerous alterations in the smooth muscle of the bladder wall (2, 7, 9, 23, 34). Not as well understood is which alterations are compensatory to maintain force output in the face of an increased obstruction produces numerous alterations in the smooth muscle of the bladder wall (2, 7, 9, 23, 34). Not as well understood is which alterations are compensatory to maintain force output in the face of an increased sensitivity of force without a concomitant decrease in the Ca\(^{2+}\) sensitivity of MLC phosphorylation. In terms of the cell biology of a smooth muscle cell, the fact that partial bladder outlet obstruction alters the isoform of myosin, as evidenced by a change in maximal shortening velocity, is of interest. In terms of the smooth muscle cells functioning together as a coordinated organ, the increase in attachment plaque-like structures after the obstruction may be important in transmission of the force signal. And last, in terms of using the Triton X-100 detergent-skinned smooth muscle cell as an experimental model, our results show that bladder smooth muscle is an excellent source of tissue for this purpose.

The differential effect of partial bladder outlet obstruction on force and MLC phosphorylation can be accounted for by at least two possibilities. The first possibility is that the obstruction produces an uncoupling between MLC phosphorylation and force development. Any cell swelling that alters the spacing between thin and thick filaments could alter the ability of actin to activate a phosphorylated myosin. Such an event has been used experimentally to inhibit contraction of smooth muscle while maintaining the ability to phosphorylate the MLC (14). Whether cell swelling or changes in the filament lattice structure occur in bladder smooth muscle after outlet obstruction is, to our knowledge, not known. However, the fact that higher levels of stress were developed in the permeabilized tissue compared with the intact tissue tends to discount this possibility.

The second possibility to account for the decrease in Ca\(^{2+}\) sensitivity of force, and not of MLC phosphorylation, is an outlet obstruction-induced loss of any regulatory pathway acting in parallel with MLC phosphorylation. We have previously shown that Ca\(^{2+}\) stimulates protein kinase C (PKC) activity in Triton X-100 detergent-skinned vascular smooth muscle (12). Additionally, we have also demonstrated that phorbol ester-, and presumably, PKC-, dependent contractions of bladder smooth muscle are attenuated after partial bladder outlet obstruction (27). Because PKC has been implicated in most smooth muscle thin filament regulatory hypotheses, it is possible that the loss of this contractile pathway contributes to the decrease in force at each submaximal Ca\(^{2+}\) concentration. Whether the decrease in Ca\(^{2+}\) sensitivity of force seen in the present study is due to a change in the filament lattice structure or loss of a parallel pathway for contractile activation cannot be definitively determined. What is definite is that partial bladder outlet obstruction has direct effects on the contractile apparatus of bladder wall smooth muscle.

By using a Triton X-100 detergent-skinned smooth muscle preparation, we were able to study the contractile apparatus without interference from receptor-mediated modulatory pathways and Ca\(^{2+}\) handling. The results obtained are, we believe, interesting on several levels. In terms of the pathophysiology of partial bladder outlet obstruction, one of the most striking findings in our study was the decrease in the Ca\(^{2+}\) sensitivity of force without a concomitant decrease in the Ca\(^{2+}\) sensitivity of MLC phosphorylation. In terms of the cell biology of a smooth muscle cell, the fact that partial bladder outlet obstruction alters the isoform of myosin, as evidenced by a change in maximal shortening velocity, is of interest. In terms of the smooth muscle cells functioning together as a coordinated organ, the increase in attachment plaque-like structures after the obstruction may be important in transmission of the force signal. And last, in terms of using the Triton X-100 detergent-skinned smooth muscle cell as an experimental model, our results show that bladder smooth muscle is an excellent source of tissue for this purpose.
Biochemical studies have clearly shown that the isoform of myosin in bladder smooth muscle changes from the faster SM-B to the slower SM-A isoform in response to partial bladder outlet obstruction (2, 9). We have also shown that this change translates into a slower maximal velocity of shortening in intact strips of bladder smooth muscle from obstructed animals (31). Our present results extend this information to directly demonstrate that maximal velocity of shortening is slower in muscle cells from animals subjected to outlet obstruction at every Ca$^{2+}$ concentration examined. Thus the activation of cross-bridge cycling in smooth muscle from the obstructed animals represents an increase in the number of contact points for the transduction of force from the contractile filaments to the cell membrane.

Rather than an increase in the number of points for transduction of force, the plaque-like structures may represent an increase in the number of gap junctions in smooth muscle from the outlet-obstructed animals. Christ and his colleagues (4) and Haeffiger and co-workers (8), using a model of rat bladder outlet obstruction, demonstrated a specific increase in connexin43. Moreover, Fry et al. (6) suggested that an increase in electrical coupling between smooth muscle cells of the human bladder may account for, in part, localized aberrant contractions. Our results do not directly address this possibility, but the time frame of our partial outlet obstruction may fit the experimental results found by at least Christ et al. (4) and Haeffiger et al. (8). These two groups used different severity of outlet obstruction, with an increase in connexin43 demonstrable after 9 h of severe and 6 wk of moderate obstruction. Our studies used a 2-wk time frame of obstruction. An increase in the number of electrical connections could account for the similar levels of force generated in an intact bladder smooth muscle preparation (31) but would not be expected to have any influence in a detergent-skinned preparation. Future studies using intact tissues should address the specific junctional components of these plaque-like structures.

A very unexpected result, at least to those who have used smooth muscle skinned fiber preparations, was the high level of stress (force/cross-sectional area) developed by the Triton X-100 detergent-skinned fibers. On average, most detergent-skinned smooth muscle preparations develop $\sim 40\% - 80\%$ of their preskingning force or stress (13, 17, 19). In our study, the Triton X-100 detergent-skinned bladder smooth muscle preparations developed nearly four times the level of stress developed by the intact smooth muscle preparation. It is possible that, even with supramaximal levels of KCl or carbachol, maximal levels of activator Ca$^{2+}$ and, therefore, stress cannot be attained in the intact preparation, whereas the direct addition of micromolar levels of Ca$^{2+}$ can be introduced into the detergent-skinned fiber. On the other hand, relative to other smooth muscles, possibly the bladder smooth muscle has a more extensive cytoskeletal structure that aids in maintaining cellular integrity after the fairly harsh exposure to Triton X-100.

Other investigators have also used skinning and permeabilizing procedures on urinary bladder smooth muscle, but the results are mixed. Using Triton X-100 detergent-skinned smooth muscle from rat urinary bladder, Arner and his co-workers (25) found that partial outlet obstruction reduced the force output compared with tissues from control animals by $\sim 25\%$. Consistent with our results, they also found that shortening velocity was significantly reduced. Kanaya (11) found similar levels of force development in a saponin-permeabilized preparation of bladder smooth muscle compared with the intact state. In these studies (11, 25) as well as others (17, 34), the Ca$^{2+}$ sensitivity of...
force ($E_{C_{50}}$) was found to be ~1 μM Ca$^{2+}$, similar to that found in our study using tissue from control animals.

As stated above, one complicating problem in the interpretation of these results is determining which alterations are compensatory to maintain normal function in the face of the obstruction and which are deleterious as a result of the obstruction. It seems intuitively obvious that the high levels of stress that can be developed by smooth muscle tissues from both animal groups, but especially the outlet-obstructed animals, helps to at least initially, maintain normal bladder function. Considering the greater the contractility ability, the better the bladders would be expected to perform, resulting in lower residual volumes. The fact that bladders from outlet-obstructed animals have a greater post-void volume, then one can assume that the force generated by the smooth muscle in the obstructed bladder is still insufficient to produce complete emptying. A similar argument could be made for the increased number of apparent attachment points for transduction of force from the contractile proteins to the membrane. In contrast, the decrease in the Ca$^{2+}$ sensitivity of force in smooth muscle from outlet-obstructed animals would seem to be a deleterious alteration. If it takes a greater level of stimulus to produce the required magnitude of force to completely empty the bladder, then one can understand why bladders from outlet-obstructed animals have a higher post-void volume. The change in myosin isoform to one with a slower actin-activated ATPase activity can be viewed in two modes: compensatory as well as deleterious. If less force is developed at any given level of stimulation, as evidenced by the decrease in the Ca$^{2+}$ sensitivity of force, then this may be countered by a longer contraction. The slower ATPase activity of the SM-A isoform, due most likely to a slower off rate, may provide a prolonged contractile event. On the other hand, if stimulation durations are not changed but it takes longer to empty the bladder, then this may lead to a greater post-void volume. Future studies aimed at comparing in vivo urodynamics and in vitro biochemistry and physiology should shed valuable light on these speculations.

Bladder function after partial outlet obstruction has been categorized as compensated (mildly dysfunctional) or decompensated (severely dysfunctional) by Levin and his colleagues (36) and by our group (28–30). As we have discussed in a previous report (31), our animal model presents bladder function consistent with a compensated state. However, our results obtained specifically from smooth muscle, as discussed above, show functional aspects that would be consistent with a compensated bladder and others that would be consistent with a decompensated bladder. The apparent discrepancy between the obstruction-induced changes as measured in whole bladder and those measured in the smooth muscle layer may have structural and temporal components. It is very possible that the obstruction-induced changes in the mucosal layer of the bladder (24) exacerbate the early but small changes in the smooth muscle, and, after longer periods of obstruction, more significant deleterious changes in the smooth muscle layer aid the pathological progression to a failing bladder. It also must be kept in mind that different species and different modes of obstruction may produce different effects on bladder smooth muscle function. Studies comparing the effects of obstruction in different animal models would be beneficial in answering this possibility.

In summary, using a Triton X-100 detergent-skinned preparation that allows the direct examination of contractile protein function in a tissue that maintains the ability to contract, we found that bladder smooth muscle from rabbits subjected to partial outlet obstruction has a reduced Ca$^{2+}$ sensitivity to force without a concomitant change in the Ca$^{2+}$ sensitivity of MLC phosphorylation. The maximal velocity of shortening was also significantly lower in smooth muscle from the obstructed animals, consistent with a change in the isoform of myosin. Electron-microscopic examination showed that the detergent-skinned cells showed excellent structural integrity and, more importantly, a significant increase in the number of sarcolemmal attachment plaquelike structures in muscle from the outlet-obstructed animals. We interpret these results to suggest that partial bladder outlet obstruction produces several alterations at the level of contractile activation and regulation that are compensatory to maintain normal force in the face of the increased resistance to flow and deleterious to bladder function that, with time, most likely aid in the deterioration of bladder function and the switch from a compensated to a decompensated state.

DISCLOSURES

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