Partial bladder outlet obstruction abolishes the receptor- and G protein-dependent increase in calcium sensitivity in rabbit bladder smooth muscle

Michaela C. Stanton, 1 Daniel Delaney, 2 Stephen A. Zderic, and Robert S. Moreland 1
1 Department of Pharmacology and Physiology, Drexel University College of Medicine, Philadelphia 19102; 2 Department of Urology, Children’s Hospital of Philadelphia, Philadelphia, Pennsylvania 19104

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Partial bladder outlet obstruction (PBOO) secondary to benign prostatic hyperplasia produces significant alterations in urinary bladder function and structure (1, 11, 31). The most common functional alteration noted clinically is an increase in the number of voids per day, a diminished volume per void, a rise in voiding pressure, and a marked increase in post void residual volume (26). Structurally, PBOO induces smooth muscle hypertrophy and a significant thickening of the connective tissue layers of the bladder (4, 31). Therefore, the goal of this current study was to test the hypothesis that PBOO decreases receptor-mediated myofilament sensitization and that the site of action is downstream from either the G proteins or PKC.

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PARTIAL BLADDER OUTLET OBSTRUCTION (PBOO) secondary to benign prostatic hyperplasia produces significant alterations in urinary bladder function and structure (1, 11, 31). The most common functional alteration noted clinically is an increase in the number of voids per day, a diminished volume per void, a rise in voiding pressure, and a marked increase in post void residual volume (26). Structurally, PBOO induces smooth muscle hypertrophy and a significant thickening of the connective tissue layers of the bladder (4, 31). Therefore, the goal of this current study was to test the hypothesis that PBOO decreases receptor-mediated myofilament sensitization and that the site of action is downstream from either the G proteins or PKC.

As stated above, numerous studies have shown that PBOO alters whole bladder function but in addition, the obstruction alters the physiology of the bladder smooth muscle cell that is responsible for maintaining the pressure needed for voiding (16, 21, 25, 27). We previously showed that one of the primary changes in the smooth muscle cell that occurs in response to outlet obstruction is a change from the typical phasic to a more tonic-like contraction (7, 27). Moreover, using a Triton X-100 detergent-skinned preparation of bladder smooth muscle, we showed that PBOO significantly reduces the Ca2+ sensitivity of force at the level of the contractile apparatus without a concomitant change in the Ca2+ sensitivity of myosin light chain (MLC) phosphorylation (25). This is significant because Ca2+-dependent MLC phosphorylation is believed to be the primary determinant of smooth muscle force development (13). If the Ca2+ dependence of force can be altered independently of MLC phosphorylation, this would suggest that other steps in the coupling of excitation to contraction may be involved. Of particular importance is the fact that intact tissues of the bladder wall that are dissected to produce a strip comprised predominantly of smooth muscle, from both control and PBOO animals, develop similar levels of maximal stress (27). Because it is well established that PBOO results in numerous intracellular changes in the smooth muscle cell (1, 7, 9, 16), the fact that similar levels of stress are developed strongly suggests that some of these changes are compensatory to counter the increased resistance to flow. This information coupled with the fact that MLC phosphorylation and force are differentially affected strongly suggests that signaling steps upstream from the contractile apparatus may be prime sites for obstruction-induced alterations.

Therefore, the goal of this current study was to test the hypothesis that steps upstream from MLC phosphorylation in the pathway leading to contraction are altered by PBOO. Specifically, we were interested in testing the hypothesis that changes in receptor- and G protein-dependent modulation of myofilament Ca2+ sensitivity occur in response to an obstruction. It is widely known that initiation of a contraction by an agonist, which acts via a receptor and G protein, produces a significant increase in the Ca2+ sensitivity of contraction compared with a contraction initiated by membrane depolarization (12, 22). The mechanism responsible for the alteration in Ca2+ sensitivity is believed to involve a Rho kinase-dependent downregulation of the MLC phosphatase resulting in higher levels of MLC phosphorylation at any given cellular [Ca2+] (22). However, other mechanisms have also been proposed to account for the enhanced sensitivity including PKC and/or the thin filament protein caldesmon (30). In this study, we addressed these questions by employing the α-toxin-permeabil-
ized fiber that allows control of the intracellular environment while maintaining intact signal transduction pathways. We present evidence suggesting that PBOO has distinct and pathophysiologically relevant effects on receptor and G protein coupling in the bladder smooth muscle cell.

MATERIALS AND METHODS

Animal Model

Male New Zealand White rabbits weighing 2–2.5 kg were used in this study. All animal studies were approved by the Institutional Animal Care and Use Committees of The Children’s Hospital of Philadelphia and Drexel University College of Medicine. PBOO was surgically performed as previously described and maintained for 2 wk (25, 27). Briefly, an 8 French catheter was inserted into the bladder through the urethra. The bladder neck was exposed via a small extraperitoneal incision, and a 4.0 silk suture was placed around the bladder neck following identification of the ureters and vas deferens. A second 8 French catheter was placed outside the urethra, and the silk suture was tied around both catheters to standardize the surgical technique. Both catheters were then removed. The rabbits were housed in metabolic cages, and data were collected for analysis of voiding frequency and volume; only those animals with a frequency greater than 30 voids/day and volumes >4 ml/void were used in these studies (26). Two weeks after partial outlet obstruction surgery, the rabbits were euthanized, the bladder was quickly removed, and it was placed in cold physiological salt solution (PSS) containing (in mM) 140 NaCl, 4.7 KCl, 1.2 MgSO4, 1.6 CaCl2, 1.2 Na2HPO4, 2.0 3-(N-morpholino)-propanesulfonic acid, 5.0 d-glucose, and 0.02 Na2-EDTA.

Tissue Preparation

Strips composed primarily of smooth muscle were prepared for measurement of isometric contraction by dissecting the middle detrusor body free of both the serosal and mucosal layers. Isometric force development was measured using a Muscle Research Station (Heidelberg, Germany). Strips (~5 mm in length and ~0.5 mm in width) were mounted between a force transducer and a stable clip in a 300-μl cuvette as previously described (32). The smooth muscle strips were allowed to equilibrate for at least 60 min in PSS using an intermittent perfusion system that completely refreshed the cuvette solution every 15 s. Tissues were stretched during the equilibration period to a length that produced a passive force of 300 μN. This passive force provided a length at which optimal active force is stabilized, the strips were exposed to a solution containing a subthreshold Ca2+-free solutions was detrimental to cellular viability. Strips were then allowed to equilibrate for 15 min in a “low EGTA relaxing solution” until a stable baseline force was reached. The strips were then perfused for 15 min in a “new EGTA relaxing solution,” and the protocol was repeated with a different [Ca2+]. The sensitivity of the strips to Ca2+ was determined by normalizing force developed at each level of Ca2+ to that attained in response to 10 μM Ca2+.

Functional status of G proteins. To determine the functional status of G proteins in the bladder smooth muscle strips, we used the GTPγS-induced release of calcium from intracellular stores and resultant force development as the index (14). The α-toxin-permeabilized strips were exposed to a solution containing a subthreshold [Ca2+] of 0.1 μM, 2 mM EGTA, 5 mM MgCl2, 20 mM imidazole (pH 6.8), 20 mM CaCl2, 71.8 mM K-acetate, and 4 mM ATP for ~30 min to reload sarcoplasmic reticular calcium stores. The Ca2+-loaded strips were then stimulated with a Ca2+-free solution containing 100 μM GTPγS and 50 μM EGTA. Any increase in force was taken as evidence of functional G proteins inducing the production of inositol trisphosphate (IP3) and the subsequent release of intracellular stores of calcium.

MLC Phosphorylation Determinations

MLC phosphorylation levels were determined in the α-toxin-permeabilized bladder strips. Strips were quick-frozen after 2 min of stimulation in a dry ice/acetone slurry containing 6% TCA (wt/vol) and 10 mM DTT. The strips were slowly thawed to room temperature, rinsed for 30 min in acetone to remove the TCA, and homogenized on ice. The homogenization buffer contained 1.0% SDS, 10% glycerol, and 20 mM DTT. The homogenates were subjected to two-dimensional SDS-gel electrophoresis followed by transfer to nitrocellulose membranes as previously described (19). Transferred proteins were visualized using Aurolate II colloidal gold stain (Amersham Pharmacia Biotech, Piscataway, NJ). MLC phosphorylation levels were quantified using a laser-scanning densitometer (model GS 800, Bio-Rad Laboratories, Richmond, CA). Values are reported as moles P, per moles of MLC and were calculated taking the density of the phosphorylated MLC as a percentage of the sum of the densities of both the unphosphorylated and monophosphorylated MLC isoforms.
Compounds and Statistics

All electrophoretic and blotting reagents were obtained from Bio-Rad Laboratories. GTP, CP, ATP, MgCl$_2$ as a 1 mg/ml solution, and EGTA were obtained from Sigma (St. Louis, MO). CaCl$_2$ was obtained as a 0.1 M standard solution (Fisher Chemicals, Pittsburgh, PA). All other reagents were of analytic grade or better and were obtained from Fisher Chemicals.

Statistical significance between means was determined using the Student’s t-test for unpaired data or ANOVA; whichever was appropriate. A P value of <0.05 was taken as significant.

RESULTS

Receptor- and G protein-dependent increases in myofilament Ca$^{2+}$ sensitivity have been shown to occur in response to most if not all agonists in most if not all smooth muscles (23). We used the α-toxin-permeabilized preparation of rabbit bladder smooth muscle from control animals to investigate potential changes in myofilament Ca$^{2+}$ sensitivity in response to increasing [Ca$^{2+}$] in the presence of carbachol and GTP compared with increasing [Ca$^{2+}$] alone. Figure 1A contains the results of these studies. Permeabilized strips of control rabbit bladder were subjected to the noncumulative addition of Ca$^{2+}$ (0.3–10 μM). After the attainment of steady-state force at each [Ca$^{2+}$], 10 μM carbachol and 10 μM GTP were added to the contracting solutions. The addition of carbachol and GTP significantly increased the sensitivity of the tissues from control animals to Ca$^{2+}$ (EC$_{50}$ Ca$^{2+}$ alone: 0.91 ± 0.03 μM; Ca$^{2+}$ plus carbachol plus GTP: 0.63 ± 0.12 μM; Fig. 1A). Consistent with previous studies using nonvascular smooth muscles, the addition of carbachol and GTP also increased the magnitude of force developed in response to a maximal [Ca$^{2+}$]. Maximal levels of force attained in response to Ca$^{2+}$ alone were 273 ± 47 mg and in response to Ca$^{2+}$ plus carbachol and GTP were 343 ± 46 mg.

Similar experiments were performed on α-toxin-permeabilized bladder smooth muscle from animals subjected to 2 wk of PBOO. The results from these experiments are shown in Fig. 1B. In stark contrast to tissues from control animals, the addition of carbachol and GTP had no significant effect on either the sensitivity or the maximal level of developed force (EC$_{50}$ Ca$^{2+}$ alone: 0.87 ± 0.12 μM; Ca$^{2+}$ plus carbachol plus GTP: 0.77 ± 0.16 μM). In fact, both curves shown in Fig. 1B are superimposable onto the concentration-response curve in response to Ca$^{2+}$ alone presented in Fig. 1A for bladder smooth muscle from control animals. Maximal levels of force attained in response to Ca$^{2+}$ alone were 166 ± 57 mg and in response to Ca$^{2+}$ plus carbachol and GTP were 178 ± 62 mg. Due to the high degree of variability in gram force measurements in the small tissues from control and obstructed animals, all results were normalized to the percent maximal force developed for each individual tissue. We previously showed that when gram force is normalized to a cross-sectional area or to muscle mass, there are no differences in contractile properties in tissues from the two animal groups (25, 27). One potentially important difference in permeabilized smooth muscles from animals subjected to PBOO compared with control is the significant elevation in force at the lowest [Ca$^{2+}$] studied, 0.3 μM.

Ca$^{2+}$-dependent MLC phosphorylation is a primary step in the initiation of a smooth muscle contraction (13). Moreover, increases in MLC phosphorylation have been suggested to underlie receptor- and G protein-dependent increases in myofilament Ca$^{2+}$ sensitivity (23). Therefore, we were interested in determining if changes in MLC phosphorylation levels could account for the carbachol- and GTP-dependent increase in force noted in bladder smooth muscle from control animals (Fig. 1A) and if a lack of change in MLC phosphorylation levels could account for the lack of effect of carbachol and GTP on force noted in bladder smooth muscle from animals subjected to PBOO (Fig. 1B). Strips of rabbit bladder smooth muscle were stimulated with various [Ca$^{2+}$] in the presence and absence of carbachol and GTP for 2 min and then frozen for quantitation of MLC phosphorylation levels. The 2-min point was chosen as it corresponds to the time at which peak force was just developed during the generation of the isometric force results. Figures 2 and 3 contain the results of these...
experiments using smooth muscles from control and obstructed animals, respectively. Increasing the [Ca\(^{2+}\)] produced an increase in MLC phosphorylation levels in smooth muscle from both control (Fig. 2A) and obstructed animals (Fig. 3A). Interestingly, although the magnitude of force development was similar in the smooth muscles from the two animal models, MLC phosphorylation levels were not significantly different in tissues from the two animal models. MLC phosphorylation values in response to Ca\(^{2+}\) plus 10 μM carbachol and 10 μM GTP were also not significantly higher in smooth muscles from obstructed (Fig. 3B) compared with those from control (Fig. 2B). This result was unexpected based on the lack of the carbachol- and GTP-dependent increase in myofilament Ca\(^{2+}\) sensitivity of force in the muscles from the obstructed animals.

There were no significant differences in MLC phosphorylation values in response to a maximal [Ca\(^{2+}\)] of 10 μM in the absence compared with the presence of carbachol and GTP. However, 10 μM carbachol and 10 μM GTP increased MLC phosphorylation levels at the lower [Ca\(^{2+}\)]. Carbachol- and GTP-induced MLC phosphorylation levels were slightly greater in the obstructed animals compared with that in control animals even though the potentiation of force was absent in the diseased model.

Receptor- and G protein-dependent changes in myofilament Ca\(^{2+}\) sensitivity are believed to result from either Rho kinase-catalyzed phosphorylation and resultant downregulation of the MLC phosphatase or PKC-catalyzed phosphorylation of CPI-17 also resulting in downregulation of the MLC phosphatase (10, 30). We were therefore interested in determining if either G protein or PKC activities were altered in smooth muscle from the animals subjected to PBOO. We used the force developed by α-toxin-permeabilized tissues with calcium-replete SR in response to GTPyS as an index of G protein function. Figure 4 shows representative tracings of such experiments. Figure 4A shows the response of bladder smooth muscle from control animals. A test contraction to 10 μM Ca\(^{2+}\) was obtained followed by relaxation in a high-EGTA-containing solution. The SR was then loaded with calcium as described in MATERIALS AND METHODS. The tissues were then switched to a zero calcium solution containing 100 μM Ca\(^{2+}\) alone. A: MLC phosphorylation values for control bladder tissues exposed to solutions containing Ca\(^{2+}\) alone. B: MLC phosphorylation values for control bladder tissues exposed to solutions containing identical [Ca\(^{2+}\)] with the addition of 10 μM carbachol and 10 μM GTP. MLC phosphorylation levels increased with increasing [Ca\(^{2+}\)]. Values are means ± SE for ≥4 determinations. *Statistical significance compared with basal at P < 0.05.

Fig. 2. Effect of carbachol and GTP on Ca\(^{2+}\)-dependent myosin light chain (MLC) phosphorylation in bladder smooth muscle from control animals. Individual tissues from control rabbit bladder were quick-frozen after 2 min of exposure to the noncumulative addition of [Ca\(^{2+}\)]. The frozen tissues were then subjected to quantitation of MLC phosphorylation levels. A: MLC phosphorylation values for control bladder tissues exposed to solutions containing Ca\(^{2+}\) alone. B: MLC phosphorylation values for control bladder tissues exposed to solutions containing identical [Ca\(^{2+}\)] with the addition of 10 μM carbachol and 10 μM GTP. MLC phosphorylation levels increased with increasing [Ca\(^{2+}\)]. Values are means ± SE for ≥4 determinations. *Statistical significance compared with basal at P < 0.05.

Fig. 3. Effect of carbachol and GTP on Ca\(^{2+}\)-dependent MLC phosphorylation in bladder smooth muscle from animals subjected to partial bladder outlet obstruction. Individual tissues from bladders subjected to 2 wk of partial bladder outlet obstruction were quick-frozen after 2 min of exposure to the noncumulative addition of [Ca\(^{2+}\)]. The frozen tissues were then subjected to quantitation of MLC phosphorylation levels. A: MLC phosphorylation values for obstructed bladder tissues exposed to solutions containing Ca\(^{2+}\) alone. B: MLC phosphorylation values for obstructed bladder tissues exposed to solutions containing identical [Ca\(^{2+}\)] with the addition of 10 μM carbachol and 10 μM GTP. MLC phosphorylation levels increased with increasing [Ca\(^{2+}\)]. Values are means ± SE for ≥4 determinations. *Statistical significance compared with basal at P < 0.05.
To test for a potential change in PKC activity in muscles from obstructed compared with control animals, we used contractions in response to PDBu. α-Toxin-permeabilized bladder smooth muscles were subjected to the cumulative addition of Ca$^{2+}$ (0.1–10 μM) and then relaxed fully. The strips were then subjected to a second cumulative Ca$^{2+}$-response curve in the presence of 1 μM PDBu. The results of these experiments are shown in Fig. 5. Cumulative [Ca$^{2+}$]-response curves obtained from obstructed animals were slightly but not significantly more sensitive than the [Ca$^{2+}$] response obtained from control animals (EC$_{50}$ control: 1.9 ± 0.1 μM; obstructed: 1.2 ± 0.1 μM). The addition of 1 μM PDBu shifted both Ca$^{2+}$-response curves to the left (EC$_{50}$ control: 1.6 ± 0.1 μM; obstructed: 1.1 ± 0.1 μM). The magnitude of the shift was similar in muscles from both sources. These results suggest that PKC activity, or at least that activated by PDBu in the presence of Ca$^{2+}$, is not altered by PBOO.

**DISCUSSION**

PBOO has been shown by numerous investigators to induce several significant alterations in the function of the whole bladder as well as in the function and regulation of the smooth muscle cells within the bladder wall (1, 2, 11, 28, 31). Whether the PBOO-induced changes in the smooth muscle cells are responsible for the functional alterations in the whole bladder, or compensatory to maintain contractility in the face of the increased resistance, is an important question that remains largely unanswered. We previously showed that PBOO depresses the response of bladder smooth muscle to an increase in cellular [Ca$^{2+}$] and that this effect is at the level of the contractile proteins (25). In this study, we addressed the possibility that PBOO alters pathway(s) upstream from the contractile proteins that modulate myofilament Ca$^{2+}$ sensitivity.

Excitation-contraction coupling in smooth muscle can be simplistically divided into two broad sets of pathways, those that directly activate the contractile proteins and those that modulate the activity of the contractile proteins. In terms of the
pathways important in modulating contractile protein activity, several groups have shown that the Ca\(^{2+}\) sensitivity of force can be significantly enhanced by a receptor- and G protein-dependent pathway (23). This is the modulatory pathway that we examined in the present study. To study the mechanism(s) responsible for enhancement of myofilament Ca\(^{2+}\) sensitivity and the apparent loss of this mechanism(s) in rabbit bladder smooth muscle, we used the well-characterized \(\alpha\)-toxin-permeabilized preparation. S. aureus \(\alpha\)-toxin forms pores in cell membranes allowing for the passive diffusion of molecules (<4,000 Da) while retaining all other larger molecules. Of particular importance is the fact that this preparation retains all signaling components in physiologically relevant concentrations. The advantage of this preparation therefore is that it allows for the study of receptor-mediated signaling pathways under controlled intracellular conditions.

The results of our studies show that PBOO significantly diminishes, if not abolishes, the receptor- and G protein-mediated increase in myofilament Ca\(^{2+}\) sensitivity. To the best of our knowledge, this represents the first demonstration of a pathophysiological state that diminished or abolished the G protein-dependent myofilament Ca\(^{2+}\) sensitization. Hypertension and asthma have been shown to enhance the G protein-dependent sensitization pathway (20, 29), but none have shown a decrease in this pathway. Additionally, we are not aware of any studies demonstrating smooth muscles that do not show an increase in myofilament Ca\(^{2+}\) sensitivity following receptor activation.

There are currently two primary hypotheses to account for the receptor- and G protein-dependent increase in myofilament Ca\(^{2+}\) sensitivity. The first is activation of Rho kinase, which catalyzes the phosphorylation of the MLC phosphatase. MLC phosphatase phosphorylation downregulates activity of the enzyme (23). The second pathway involves activation of PKC and the subsequent phosphorylation of a MLC phosphatase inhibitor, CPI-17. CPI-17 phosphorylation activates the inhibitor with the resultant downregulation of MLC phosphatase activity (30). Thus the end point for both pathways is inhibition of the MLC phosphatase resulting in a greater level of MLC phosphorylation at any given [Ca\(^{2+}\)] and therefore presumably force.

In this study, we tested whether PBOO inhibited G protein function. If one accepts the assumption that a GTP\(_S\)-induced contraction is mediated by activation of G proteins, generation of IP\(_3\), and release of calcium from the SR, then our results would suggest that PBOO does not alter G protein activity or at least not that which is involved in IP\(_3\) generation. The methodology we used has been previously employed to study the IP\(_3\) pathway in \(\alpha\)-toxin-permeabilized smooth muscles (14) and as such we believe our assumptions are valid. One point of potential interest is the small but significant increase in force developed in bladder smooth muscle from the PBOO animals in response to what was assumed to be a subthreshold [Ca\(^{2+}\)] in the presence of low EGTA (Fig. 4B). This is consistent with the elevated force noted in Fig. 1B at the lowest [Ca\(^{2+}\)]. Whether this is due to a selective increase in Ca\(^{2+}\) sensitivity at the lowest end of the concentration-response relationship or due to a specific alteration in contractile regulation is not known. Intact smooth muscles from animals subjected to PBOO do not have higher levels of basal tone, although they do express a significant increase in spontaneous activity suggestive of a change in calcium handling or calcium responsiveness (27). In terms of the present study, any alteration in response to PBOO that diminishes or abolishes the pathway responsible for myofilament Ca\(^{2+}\) sensitization must be downstream from the membrane-bound receptor-coupled G proteins.

We also investigated the potential loss or alteration of the PKC-mediated pathway that results in an increase in myofilament Ca\(^{2+}\) sensitization. We used PDBu-stimulated contraction as an index of the presence of PKC available for activation. The addition of PDBu increased the sensitivity of the permeabilized tissues from both control and PBOO animals to Ca\(^{2+}\). This was a surprising result to us as we showed in a preliminary report that intact bladder smooth muscles from PBOO animals do not respond to the addition of PDBu, whereas intact tissues from control animals respond to PDBu with the typical slowly developing contraction (24). It is possible that we were studying different isoforms of PKC in these two studies. In the present study, we contracted the permeabilized tissues with increasing [Ca\(^{2+}\)] in the presence and absence of PDBu. In the intact tissues, we added PDBu to the tissues in a bathing solution of normal calcium. Intracellular [Ca\(^{2+}\)] most likely did not increase in the intact tissues. If this possibility is correct, then it would suggest that in the current study we are examining the calcium-dependent class of PKC, whereas in the intact tissue report (24), we were examining the calcium-independent or possibly the atypical classes.

On the other hand, it is possible that PBOO induces molecular alterations in the bladder smooth muscle that result in the loss of the PKC-dependent pathway important in increasing the Ca\(^{2+}\) sensitivity of a contraction but not that which is responsible for the direct activation of a contraction. We previously showed that PDBu-induced contractions in intact vascular smooth muscle are not dependent on either Ca\(^{2+}\) or MLC phosphorylation (8). The PKC-dependent pathway activated in this situation (8) would be expected to be different than that activated to alter a Ca\(^{2+}\)-dependent contraction through changes in MLC phosphatase activity.

As discussed above, the final common end point for both the G protein-dependent Rho kinase-mediated or PKC-mediated pathways for enhancement of myofilament Ca\(^{2+}\) sensitivity is downregulation of the MLC phosphatase. As such, an alteration in the stimulated levels of MLC phosphorylation should be evident in smooth muscle from the PBOO animals compared with those from control animals. We did not find any decreases in carbachol plus GTP-induced MLC phosphorylation levels in smooth muscle from PBOO animals compared with those from control. In fact, if there was a change, it was in the opposite direction such that MLC phosphorylation levels in response to carbachol plus GTP tended to be higher in tissues from the PBOO animals compared with those from control. One caveat that must be mentioned is that due to the reasonably small changes in MLC phosphorylation that occur during a typical contraction/relaxation cycle (27), precise quantitative measurements are more difficult than in other smooth muscles that show large changes in phosphorylation (8, 18). However, what is certain is that a decrease in MLC phosphorylation was not noted in tissues from PBOO animals, although the carbachol plus GTP-dependent increases in force were abolished. Although this is an unusual finding, it may reflect the fact that we are studying a tissue that has undergone a change from a predominantly phasic contractile profile to one
that is predominantly tonic (7, 27). Woodsome et al. (30) found differential effects of PDBu and GTPγS on CPI-17 phosphorylation and the resultant potential increase in levels of MLC phosphorylation and force in phasic compared with tonic smooth muscles. It is possible that bladder smooth muscles from animals subjected to PBOO retain some contractile properties associated with a phasic tissue while gaining some properties more associated with a tonic tissue. Further examination of the time course of the PBOO-induced alterations and correlating these alterations with protein expression and contractile regulation are needed to address this possibility.

Bladders subject to PBOO undergo a myriad of changes at the molecular, cellular, and physiological level. Changes in protein isoforms have already been reported to account for some changes in bladder function and contractile ability (4, 7, 21). It would be expected that changes in the expression of other proteins would occur to compensate for the cellular and physiological changes observed in a dysfunctional bladder or in fact be responsible for the dysfunction. The loss of the carbachol- and GTP-dependent enhancement of myofilament Ca2+ sensitivity could be seen as a change that could, in part, induce bladder dysfunction. If any change in the smooth muscle cell significantly reduces the amount of contractile force development per stimulation, then both the voiding time and voiding volume in the whole animal would be decreased. On the other hand, it has been shown that cholinergic innervation increases following PBOO in guinea pigs (5) and rats (15). This was suggested to induce a state of receptor or contractile desensitization that would diminish contractile responses to carbachol. In contrast, Burnstock’s group (3) saw a decrease in cholinergic input to the bladder following PBOO in rabbits. This latter observation would be more consistent with our previous demonstration that bladders from rabbits subjected to PBOO showed increased resistance to contractile desensitization (27).

An intriguing although speculative hypothesis stems from work initiated by Levin’s group (11). They proposed that following PBOO, the significant thickening of the bladder wall due to hypertrophy in conjunction with damage to perfusing blood vessels may induce a state of ischemia. The receptor- and G protein-dependent increase in myofilament Ca2+ sensitivity significantly increases the cellular utilization of ATP. The increase in ATP utilization is from the increase in actin-activated myosin ATPase activity in response to the increased levels of MLC phosphorylation. Thus two sites of ATP usage are potentially increased. If the ischemia is sufficiently severe, then maintained levels of cellular ATP may not be high enough to sustain the increased demand. Although this is in some ways an attractive hypothesis to account for the present results, we showed in vascular smooth muscle that ATP levels must fall to extremely low levels before the cross-bridge activity is affected (6).

In summary, using an α-toxin-permeabilized preparation of rabbit bladder smooth muscle, we examined the potential effect(s) of PBOO on signaling steps important in excitation-contraction coupling. We demonstrated that PBOO significantly diminished or abolished the well-described receptor- and G protein-dependent increase in myofilament Ca2+ sensitivity. This effect was not mediated by a loss in G protein function, at least not the G protein activity responsible for generation of IP3 and release of intracellular calcium. It was also not associated with a decrease in Ca2+-dependent isoforms of PKC that can be activated by PDBu. The loss of the G protein-dependent enhancement of myofilament Ca2+ sensitivity was also not associated with a decrease in MLC phosphorylation levels. In fact, if anything, MLC phosphorylation levels were increased following stimulation of bladder smooth muscle from the PBOO animals with carbachol and GTP. We propose two hypotheses that are consistent with our results. First, is that PBOO uncouples MLC phosphorylation from force; this is consistent with our present study in which the G protein-dependent enhanced force is abolished while MLC phosphorylation levels are unaffected. This is also consistent with a previous report from our group (25) demonstrating that PBOO decreases the calcium sensitivity of force in a Triton X-100-skinned fiber without a concomitant change in the calcium sensitivity of MLC phosphorylation. The second hypothesis is that PBOO downregulates a specific isoform of PKC, one that is important in the G protein-mediated enhancement of Ca2+ sensitivity but not one that mediates Ca2+ and PDBu and dependent contractions. This hypothesis would allow for the apparently disparate results found in this present study where PDBu increased Ca2+-dependent contractions of bladder smooth muscle from PBOO animals with a previous report from our group (24) showing that intact bladder smooth muscle from PBOO animals did not respond to PDBu stimulation. Future studies delineating whether either or both of these hypotheses are correct would be of interest.

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