Length-dependent regulation of basal myosin phosphorylation and force in detrusor smooth muscle

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Ratz, Paul H. and Amy S. Miner. Length-dependent regulation of basal myosin phosphorylation and force in detrusor smooth muscle. Am J Physiol Regul Integr Comp Physiol 284: R1063–R1070, 2003. First published December 19, 2002; 10.1152/ajpregu.00596.2002.—Urinary bladder (detrusor) smooth muscle is active in the absence of an external stimulus. Tone occurs even “at rest” during the filling phase, and it is elevated in patients with overactive bladder. This study examined the role of muscle length on tone and the level of basal myosin light chain phosphorylation (MLC20P). MLC20P was 23.9 ± 1% (n = 58) at short lengths (zero preload; Ls). An increase in length from Ls to the optimal length for contraction (Lo) caused a reduction in MLC20P to 15.8 ± 1% (n = 49). Whereas 10 μM staurosporine reduced MLC20P at Ls, 1 μM staurosporine, a Ca2+i-free solution, and inhibitors of MLC kinase, protein kinase C (PKC) and RhoA kinase (ROK) did not. However, 1 μM staurosporine and inhibitors of ROK inhibited MLC20P and tone at Ls. These data support the hypothesis that a Ca2+i-independent kinase, possibly ZIP-like kinase, regulates MLC20P at Ls, whereas in detrusor stretched to Lo, additional kinases, such as ROK, participate.

rabbit urinary bladder; muscle stretch; preload; signal transduction; myogenic tone

THE FUNCTION OF THE URINARY bladder is to store and expel urine. Detrusor smooth muscle is innervated by the autonomic nervous system, and the primary stimulus producing detrusor contraction leading to elimination of urine is acetylcholine released on activation of cholinergic motor nerves (6, 8, 9). However, Stewart (42) demonstrated a century ago that the bladder is not completely “at rest” when neurogenic stimuli are absent during the filling phase. Rather, detrusor exhibits spontaneous rhythmic contractions (tone), reflecting intrinsic rather than neurogenic activity (1, 2, 4, 48). Detrusor from patients with overactive bladder, a disorder involving involuntary detrusor contractions that occur during bladder filling (12), displays enhanced contractile tone (21).

To contract smooth muscle, many stimuli activate subcellular signaling systems that mobilize Ca2+i from extracellular and intracellular stores, resulting in an elevation in intracellular free Ca2+i ([Ca2+i]) (15). Elevated [Ca2+i] increases myosin light chain (MLC) kinase activity, MLC phosphorylation, and cross-bridge cycling, resulting in elevations in contractile force (41). This general scheme has been shown to play a primary regulatory role in vascular smooth muscle, but whether it plays the principal role in regulation of detrusor smooth muscle remains to be determined. In a recent review, Hypolite et al. (17) provide tantalizing data indicating that MLC phosphorylation is elevated in resting detrusor, and this proposal was recently confirmed (18). However, what cellular mechanisms cause this high basal MLC phosphorylation, and whether the high basal MLC phosphorylation is dependent on muscle length, is not known. We therefore examined the effect of muscle length on basal MLC phosphorylation. Using selective pharmacological probes, we also tested the hypothesis that basal MLC phosphorylation and basal contractile force (tone) are caused by the basal activity of MLC kinase or other ser/thr kinases.

MATERIALS AND METHODS

Tissue preparation. Tissues were prepared as described previously (33, 40). Whole bladders and femoral arteries from adult female New Zealand White rabbits were removed immediately after death with pentobarbital sodium. Bladders and arteries were washed several times and stored in cold (0–4°C) physiological salt solution (PSS) composed of (in mM) 140 NaCl, 4.7 KCl, 1.2 MgSO4, 1.6 CaCl2, 1.2 Na2HPO4, 2.0 MOPS (adjusted to pH 7.4 at either 0 or 37°C, as appropriate), 0.02 Na2-EDTA (to chelate trace heavy metals), and 5.6 dextrose. High-purity (17 MΩ) water was used throughout. For clarity in RESULTS, PSS will be referred to as a “Ca2+i-containing solution” while PSS with no CaCl2 and the addition of 1 mM EGTA to chelate Ca2+i as a “Ca2+i-free solution.” Longitudinal detrusor muscle strips free of underlying urothelium were cut from the wall of the bladder above the trigone, and endothelium was removed from femoral arteries, which were cut into 3-mm-wide rings. Muscle tissues were incubated in aerated PSS at 37°C in water-jacketed tissue baths (Radnoti Glass Technology, Monrovia, CA). Tissues that were to be stretched to their optimum length for muscle contraction (Lo) were secured by small clips to a micrometer for length adjustments and a force transducer (Harvard Bioscience, Holliston, MA and Radnoti Glass Technology, Monrovia, CA) for measurement of isometric contraction.

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Contraction of isolated detrusor strips and treatment of strips maintained at a length producing zero preload (Lz). Isometric contraction was measured as described previously (34, 40). Voltage signals were digitized (model DIO-DAS16, ComputerBoards, Mansfield, MA), visualized on a computer screen as force (g), and stored for analyses. All data analyses were performed by using a multichannel data integration program (DASYLab, TasyTec USA, Amherst, NH). Tissues were equilibrated for a minimum of 30 min suspended without tension between micrometer and force transducer. Some tissues were then stretched to Lz by using an abbreviated length-force determination in which the optimal force for muscle contraction (F0) produced by 110 mM KCl at Lz was obtained (14, 37, 47). Tissues were incubated in a Ca2+-free solution and subjected to a quick-release protocol to obtain passive force values (14). To reduce tissue-to-tissue variability, subsequent contractions were reported as normalized to F0/F0c, where F is contractile force. Detrusor produces rhythmic contractions under basal conditions, and basal contractile tone is defined as the average rhythmic contractile force produced over a period of ~3 min minus passive force (39). To determine the effect of a drug on basal tone, control basal tone was recorded for 10 min, tissues were exposed to a given concentration of the drug, and basal tone was recorded for an additional 30 min. The average force produced at steady state (30 min after drug addition) was normalized to the average force produced just before addition of a drug. The protocol used to stretch muscle strips to Lz lasted ~2 h and included several solution changes involving addition and washout of KCl. Muscle strips studied at short muscle lengths (Ls) were exposed to the same solutions (including KCl), and number of solution changes over the same duration of time. Thus muscle strips at Ls and Lz were treated identically except that muscle strips at Ls were stretched and maintained at a preload (passive force) ~9% of F0, whereas most muscle strips maintained at Lz were never stretched. However, for those experiments in which tissues were rapidly stretched from Ls to Lz, Lz was first obtained, and tissues were then shortened (unstretched) completely to Ls for at least 30 min before they were stretched rapidly back to Lz.

MLC phosphorylation. The degree of MLC phosphorylation was measured as described previously (19, 37). Tissues were quick frozen in an acetone-dry ice slurry, slowly warmed to room temperature, dried, weighed, and homogenized on ice in 8 M urea, 2% Triton X-100, and 20 mM dithiothreitol. Isoelectric variants of the 20-kDa MLCs were separated by two-dimensional (isoelectric focusing-SDS) PAGE followed by Western blot, visualized by colloidal gold stain, and the relative amounts of phosphorylated and nonphosphorylated MLCs were quantified by digital image analysis.

Drugs. HA-1077, Y-27632, and GF-109203X were made as stock solutions in distilled water. Trifluoperazine (TFP), wortmannin, and staurosporine were dissolved in DMSO, which was added at a final concentration of 0.1%. All other drugs were from Calbiochem, Alexis, or Sigma Chemical.

Statistics. Analysis of variance and the Student-Newman-Keuls test, or the t-test, was used where appropriate to determine significance, and the null hypothesis was rejected at P < 0.05. The population sample size (n value) refers to the number of animals, not the number of tissues.

RESULTS

Dependency of MLC phosphorylation on muscle stretch. Detrusor maintained at short muscle lengths (Ls; see MATERIALS AND METHODS) displayed high basal MLC phosphorylation (Fig. 1A). This high level of MLC phosphorylation was more than twofold greater than the basal level produced by ureter or artery also maintained at Lz (Fig. 1A). Interestingly, stretching detrusor strips to Ls for at least 30 min reduced the degree of MLC phosphorylation by approximately one-half (Fig. 1B, Lz-to-Ls). Moreover, when detrusor was stretched from Ls to Lz to cause a reduction in MLC phosphorylation, then rapidly (1 s) released back to Lz and maintained at Lz for 30 min, MLC phosphorylation significantly increased (Fig. 1B, Lz-to-Ls).

Regional difference in the degree of basal MLC phosphorylation. Single muscle strips were dissected from dome to trigone and cut in half, dividing them into upper detrusor (the half closest to the dome) and lower detrusor (the half closest to the trigone). Upper detrusor exhibited greater levels of MLC phosphorylation at Lz and Ls compared with lower detrusor (Fig. 2A). Moreover, in tissues stretched to Ls, the degree of contractile tone produced by upper detrusor was greater than that produced by lower detrusor (Fig. 2B) despite the fact that tissues from both upper and lower detrusor were stretched by the same degree (i.e., passive-to-active force ratios were identical in upper and lower detrusor; Fig. 2C). Upper detrusor was used in all subsequent studies.

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from control (Fig. 3F). Contractile tone is reportedly not reduced by atropine (2). Our data confirm this observation (Fig. 3F) and indicate that basal MLC phosphorylation in detrusor at $L_o$ (Fig. 3B) and $L_o$ (Fig. 3A) also was not reduced by atropine.

Effect of inhibitors of MLC kinase on basal MLC phosphorylation and contractile tone. The study examined the ability of several different pharmacological agents to reduce MLC phosphorylation in tissues maintained at $L_o$. All agents were dissolved in distilled water or DMSO. Triptolide (TFP), a calmodulin blocker, and wortmannin both effectively inhibit MLC kinase activity at 50 and 3 μM, respectively (5, 16, 27, 30, 46). ML-9 is also a MLC kinase inhibitor, with a $K_i$ value for smooth muscle MLC kinase of ~4 μM (38). However, ML-9 may also inhibit RhoA kinase (ROK), because its potency at relaxing rat aorta correlates with its ability to displace an analog of Y-27632 from its binding site on ROK (45). At 50, 10, and 3 μM, respectively, TFP, ML-9, and wortmannin did not reduce basal MLC phosphorylation in detrusor strips at $L_o$ (Fig. 4A).

In tissues stretched to $L_o$, all three agents reduced tone. TFP and wortmannin nearly abolished tone, but at the concentration used, ML-9 produced a modest 25–30% inhibition of tone (Fig. 4, C–F). Concomitant with the large inhibition of tone, TFP and wortmannin reduced the degree of basal MLC phosphorylation in tissues at $L_o$ from the control value of ~16 to ~10%
lation by inhibited the PE-induced increase in MLC phosphorylation. The finding that TFP did not inhibit MLC phosphorylation produced at $L_z$ suggested that MLC kinase did not play a part in generating the high basal phosphorylation. Whether this phosphorylation was not inhibited because it reflected a very low MLC phosphorylation turnover in tissues at $L_o$, was not determined. To address this possibility, tissues at $L_o$ were exposed to TFP for 15 min, released to $L_o$ for 1 min, and quick frozen to measure the degree of MLC phosphorylation (see Fig. 6B). For a comparison, other tissues were maintained at $L_o$ (not released to $L_z$; see Fig. 6A). Experimental results shown in Fig. 1B revealed that MLC phosphorylation increased to $\sim 21\%$ in tissues released from $L_o$ to $L_z$. The present experiment showed that this increase occurred within 1 min and that the increase was not inhibited by TFP (Fig. 6C).

Lack of effect of the calmodulin antagonist, TFP, on the ability of detrusor to produce an increase in MLC phosphorylation when released from $L_o$ to $L_z$. The inability of TFP, ML-9, and wortmannin to reduce MLC phosphorylation in detrusor at $L_z$ was a surprising finding. Thus, for a comparison, the ability of TFP, wortmannin and ML-9 to inhibit increases in MLC phosphorylation produced by stimulation of $\alpha$-adrenergic receptors by 1 $\mu$M phenylephrine (PE) in another smooth muscle type (femoral artery) that also was maintained at $L_z$ was examined. Moreover, the ability of these agents to inhibit MLC phosphorylation produced by 100 $\mu$M bethanechol (BE; muscarinic receptor agonist) was examined in detrusor. Each agent inhibited the PE-induced increase in MLC phosphorylation by $\geq 50\%$ (Fig. 5A). This was in sharp contrast to the effect of TFP and wortmannin on the muscarinic receptor-induced increase in MLC phosphorylation in detrusor at $L_z$. Although the muscarinic receptor agonist BE significantly elevated MLC phosphorylation from a basal level of $\sim 24\%$ to a stimulated level of $\sim 37\%$, neither TFP nor wortmannin produced a significant reduction in this stimulated MLC phosphorylation (Fig. 5B). However, in detrusor tissues stretched to $L_o$ and stimulated with 100 $\mu$M BE, both force (Fig. 5C) and MLC phosphorylation (Fig. 5D) were significantly reduced by TFP and wortmannin.

Effect of staurosporine and a $Ca^{2+}$-free solution on basal MLC phosphorylation and contractile tone. When used at 1 $\mu$M for 50 min, staurosporine, a general inhibitor of Ser/Thr kinases (7, 13, 31, 44, 49, 51), did not reduce the degree of basal MLC phosphorylation in detrusor at $L_z$ (Fig. 7A) but nearly abolished both basal MLC phosphorylation (Fig. 7B) and tone (Fig. 7C) in (Fig. 4B). The modest decrease in tone produced by ML-9 did not correspond with a measurable decrease in the extent of basal MLC phosphorylation (Fig. 4B).

Effect of inhibitors of MLC kinase on contractile agonist-induced increases in MLC phosphorylation and force. The inability of TFP, ML-9, and wortmannin to reduce MLC phosphorylation in detrusor at $L_z$ was a surprising finding. Thus, for a comparison, the ability of TFP, wortmannin and ML-9 to inhibit increases in MLC phosphorylation produced by stimulation of $\alpha$-adrenergic receptors by 1 $\mu$M phenylephrine (PE) in another smooth muscle type (femoral artery) that also was maintained at $L_z$ was examined. Moreover, the ability of these agents to inhibit MLC phosphorylation produced by 100 $\mu$M bethanechol (BE; muscarinic receptor agonist) was examined in detrusor. Each agent inhibited the PE-induced increase in MLC phosphorylation by $\geq 50\%$ (Fig. 5A). This was in sharp contrast to the effect of TFP and wortmannin on the muscarinic receptor-induced increase in MLC phosphorylation in detrusor at $L_z$. Although the muscarinic receptor agonist BE significantly elevated MLC phosphorylation from a basal level of $\sim 24\%$ to a stimulated level of $\sim 37\%$, neither TFP nor wortmannin produced a significant reduction in this stimulated MLC phosphorylation (Fig. 5B). However, in detrusor tissues stretched to $L_o$ and stimulated with 100 $\mu$M BE, both force (Fig. 5C) and MLC phosphorylation (Fig. 5D) were significantly reduced by TFP and wortmannin.

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detrusor stretched to \( L_o \). Similarly, incubation of detrusor in a \( \text{Ca}^{2+} \)/H11001-free solution for 30 min greatly reduced basal MLC phosphorylation and tone in tissues at \( L_o \), but it had no effect on the high basal level of MLC phosphorylation of detrusor at \( L_z \) (Fig. 7, \( D-F \)). Interestingly, however, 10 \( \mu \)M staurosporine did reduce basal MLC phosphorylation in detrusor at \( L_z \) (Fig. 7A, crosshatched bar).

**Effect of inhibitors of ROK and PKC on basal MLC phosphorylation and contractile tone.** Y-27632 and HA-1077 have been used extensively to block ROK activity in intact tissues (3, 19, 22, 43, 45). Both agents are highly effective ROK inhibitors, and Y-27632 has a high degree of selectivity for inhibition of ROK (5, 43). GF-109203X at 1 \( \mu \)M is an effective inhibitor of conventional and novel isoforms of PKC (11, 25). None of these agents reduced basal MLC phosphorylation produced in detrusor at \( L_z \) (Fig. 8A). However, the ROK inhibitors reduced both basal MLC phosphorylation (Fig. 8B) and tone (Fig. 8, \( C \) and \( E \)) in detrusor at \( L_z \). The PKC inhibitor GF-109203X produced a slight reduction in the average values of MLC phosphorylation and tone in tissues stretched to \( L_o \), but this apparent reduction was not significantly different from the control values (Fig. 8, \( B \) and \( E \)). When individual responses were analyzed, some tissues displayed an apparent but small reduction in tone in response to 1 \( \mu \)M GF-109203X (Fig. 8D).

![Fig. 6. Comparison of the effect of TFP, a calmodulin blocker and inhibitor of MLC kinase, on basal MLC phosphorylation (C) in detrusor at \( L_o \), and in detrusor released from \( L_o \) to \( L_z \) for 1 min (1'). Examples of basal tone and the effects of TFP are shown in A and B, and an example of the release from \( L_o \) to \( L_z \) is shown in B. Freeze, quick freeze in acetone and dry ice (see MATERIALS AND METHODS); 15', 15 min. Values in C are means ± SE; \( n \) values (no. of animals) are shown in parentheses. * \( P < 0.05 \) compared with \( L_o \).](http://ajpregu.physiology.org/)

![Fig. 7. Effect of the general Ser/Thr kinase inhibitor staurosporine (STSP; A, B and C) and a \( \text{Ca}^{2+} \)-free solution (D, E, and F) on basal MLC phosphorylation in detrusor at \( L_z \) (A and D) and \( L_o \) (B and E) and on basal tone in detrusor at \( L_z \) (C and F). Values are means ± SE; \( n \) values (no. of animals) are shown in parentheses. * \( P < 0.05 \) compared with control tissues.](http://ajpregu.physiology.org/)

![Fig. 8. Effect of inhibitors of RhoA kinase (1 \( \mu \)M and 3 \( \mu \)M Y-27632, and 10 \( \mu \)M HA-1077) and PKC\( \varepsilon \) (1 \( \mu \)M GF-109203X) on basal MLC phosphorylation in detrusor at \( L_z \) (A and B) and \( L_o \) (C and D), and on basal tone in detrusor at \( L_z \) (E). Examples of basal tone and the effects of 1 \( \mu \)M Y-27632 and GF-109203X are shown in C and D, respectively. Drugs were added at 10 min. Values in A, B, and E are means ± SE; \( n \) values (no. of animals) are shown in parentheses. \( F_{\text{postdrug}}/F_{\text{predrug}} \) average force produced at steady state (30 min after drug addition) was normalized to the average force produced just before addition of a drug. * \( P < 0.05 \) compared with control tissues, which did not receive a drug.](http://ajpregu.physiology.org/)
DISCUSSION

The present study was designed to test the hypotheses that MLC phosphorylation is responsible for detrusor basal tone, and that MLC kinase causes basal MLC phosphorylation in detrusor. Results from this study support the former, but they call into question a role for MLC kinase in causing basal MLC phosphorylation in detrusor. The most surprising finding in this study was that MLC phosphorylation in detrusor maintained at $L_o$ (a short muscle length where passive force was zero) was elevated compared with the level of MLC phosphorylation in detrusor stretched to $L_o$ and that this high basal MLC phosphorylation at $L_o$ was not reduced by any agent tested except 10 $\mu$M staurosporine. These data support the hypothesis that several kinases regulate basal MLC phosphorylation in detrusor, and that the degree of detrusor stretch regulates basal MLC phosphorylation via modulation of kinase activity or substrate availability.

The bladder above the trigone region does not display gross demarcations permitting identification of discrete regions. However, detrusor strips isolated from upper regions of detrusor produce stronger contractions than do strips isolated from lower regions (23). Moreover, upper detrusor produces a higher level of basal MLC phosphorylation than does lower detrusor (18). The present study extends these observations by showing that the degree of basal tone correlates with the degree of basal MLC phosphorylation when upper and lower detrusor are compared. That is, both MLC phosphorylation and basal tone were higher in upper detrusor than lower detrusor when tissues from both regions were stretched to $L_o$ (see Fig. 2). Thus these results taken together support the hypothesis that the degree of MLC phosphorylation regulates the degree of spontaneous detrusor contraction. However, the present study also showed that the degree of basal MLC phosphorylation from both regions could be nearly doubled simply by reducing muscle length such that the muscles were at zero preload ($L_z$; see Fig. 2A). Thus muscle length (or preload) contributed greatly to the absolute degree of MLC phosphorylation.

The very high level of MLC phosphorylation produced by detrusor at $L_z$ may be a unique feature of detrusor smooth muscle, because at least two other smooth muscle types, ureter and artery, did not demonstrate these high levels of basal MLC phosphorylation at $L_z$ (see Fig. 1A). Perhaps the most significant finding was that the increase in the degree of MLC phosphorylation when tissues were shortened (from $L_o$ to $L_z$) was not caused by an increase in the activation state of the kinases responsible for producing basal MLC phosphorylation in tissues stretched to $L_o$. This conclusion is based on the fact that the very high basal MLC phosphorylation produced in tissues at $L_z$ could not be reduced by a $\text{Ca}^{2+}$-free solution; inhibitors of MLC kinase [TFP, wortmamin, and ML-9 (16, 27, 30, 38)] and ROK [Y-27632 and HA-1077 (5, 43, 45)]; an inhibitor of conventional and novel PKC isotypes [GF-109203X (10, 11, 25)]; or 1 $\mu$M staurosporine, which is known to inhibit conventional and novel PKC isotypes (26, 44), integrin-linked kinase [ILK (7)], and p21-activated kinase [PAK (51)]. This rules out not only many known kinases that can use the 20-kDa MLC as a substrate (31, 50), including MLC kinase, $\text{Ca}^{2+}$/calmodulin-dependent kinase II, PKC-\(\alpha\), ILK, and PAK, but also ROK and PKC-\(\delta\), which can phosphorylate both MLC and proteins associated with the catalytic subunit of MLC phosphatase to reduce MLC phosphatase activity and elevate MLC phosphorylation (10).

Of all the agents used in this study, only 10 $\mu$M, but not 1 $\mu$M, staurosporine produced a significant inhibition of MLC phosphorylation in detrusor at $L_z$. Interestingly, 10 $\mu$M but not 1 $\mu$M staurosporine inhibits ZIP-like kinase (31), a protein found in rabbit bladder and identified recently as the endogenous smooth muscle myosin phosphatase-associated kinase (24) that can cause $\text{Ca}^{2+}$-independent elevations in MLC phosphorylation (31). On the basis of these results, it is tempting to speculate that the basal MLC phosphorylation produced in detrusor at $L_z$ is caused by ZIP-like kinase and that the level of ZIP-like kinase activity in detrusor is dependent on the degree of muscle stretch. However, whether the ZIP-like kinase, PKC-\(\xi\) (11), an atypical PKC isotype that, like ZIP-like kinase, can be inhibited by 10 $\mu$M staurosporine (26), or another $\text{Ca}^{2+}$-independent protein kinase plays a role in causing the high basal MLC phosphorylation in detrusor at $L_z$ remains an issue to be addressed in future studies. Moreover, our data cannot rule out the hypothesis that MLC phosphatase activity was reduced at $L_z$.

The level of MLC phosphorylation produced by detrusor stretched to $L_z$ was reduced by ROK inhibitors, an inhibitor of calmodulin (TFP), a $\text{Ca}^{2+}$-free solution, and an inhibitor of MLC kinase (wortmannin). However, another MLC kinase inhibitor, ML-9, although strongly reducing the PE-stimulated increase in MLC phosphorylation in femoral artery, had no effect on basal MLC phosphorylation in detrusor. Wortmannin is also an inhibitor of phosphatidylinositol-3-kinase, an enzyme recently shown to play a role in regulation of $\text{Ca}^{2+}$ entry in vascular smooth muscle (32). These results indicate that ROK and a $\text{Ca}^{2+}$/calmodulin-dependent enzyme participated in the regulation of MLC phosphorylation in detrusor stretched to $L_o$, but they also raise the possibility that MLC kinase contributed minimally to MLC phosphorylation. Although 1 $\mu$M GF-109203X does inhibit muscarinic receptor-stimulated detrusor contraction (36), the present study demonstrated no significant effect of 1 $\mu$M GF-109203X on basal MLC phosphorylation or tone. Thus conventional and novel PKC isotypes, such as PKC-\(\alpha\) and PKC-\(\delta\) (10, 11, 25), did not appear to contribute to regulation of basal MLC phosphorylation in detrusor stretched to $L_o$.

Precisely how the degree of muscle stretch altered the mechanism regulating MLC phosphorylation was not determined in this study. However, TFP and wortmannin inhibited both basal and BE-stimulated MLC phosphorylation only in tissues at $L_z$, not in tissues at $L_o$. This suggests that muscle length regulated a mechanism downstream from agonist-induced cell signaling
could not be ruled out. At longer muscle lengths ($L_o$), basal MLC phosphorylation was high (~24%), but this phosphorylation was likely not caused by MLC kinase, conventional and novel PKC isoatypes, ROK, PAK, or ILK; however, ZIP-like kinase and atypical PKC isoatypes could not be ruled out. At longer muscle lengths ($L_o$), where preload was ~9% of $F_o$, the level of basal MLC phosphorylation was significantly lower (~16%), and other protein kinases (such as ROK) participated in regulating MLC phosphorylation and tone. Detrusor from patients with overactive bladder display enhanced spontaneous contractile tone (12, 21). Thus a detailed understanding of mechanisms underlying regulation of detrusor contractions should provide insights into the identification of potential new therapies directed toward alleviating symptoms of overactive bladder. The present study represents a step in this direction.

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