Role of myosin light chain kinase in regulation of basal blood pressure and maintenance of salt-induced hypertension

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SYSTEMIC BLOOD PRESSURE is regulated by complex neurohumoral and mechanical signals that affect cardiac output, extracellular fluid volume, and vascular resistance, which arises from the contraction of arterial smooth muscle (3–5, 8, 21). Elevated blood pressure primarily occurs through a canonical mechanism that emphasizes a central role of the renin-angiotensin-aldosterone system in regulating volume expansion and systemic vascular resistance (8, 20, 23). Although it has been recognized for the past several decades that alteration in arterial wall structure, arterial smooth muscle hypersensitivity to vasoconstrictors, depressed responsiveness to vaso dilators, and impaired endothelium function can increase vascular resistance and blood pressure, the contributions of vascular contractility to physiological blood pressure as well as hypertension have not been sufficiently tested (36). More recent reports suggest an important role of vascular contractility in high blood pressure with evidence that abnormalities of the vascular smooth muscle (VSM) contractile state are sufficient to cause disorders in the regulation of blood pressure, including hypertension (10, 23, 25, 26). However, the mechanisms regulating vascular contractility in relation to blood pressure are not well defined, and genetic models of impaired vascular contractility are rare.

VSM contractility is regulated by a network of signaling pathways directed to the molecular motor myosin (15, 16, 33, 34). Phosphorylation of the regulatory light chain (RLC) of smooth muscle myosin activates actomyosin Mg-ATPase resulting in cross-bridge cycling and force development in smooth muscle. The extent of RLC phosphorylation, regulated by multiple signaling pathways that impinge on both Ca2+/calmodulin-dependent myosin light chain kinase (MLCK) and myosin light chain phosphatase (MLCP), is thought to be the primary determinant for the production of force (16, 33, 34). However, other Ca2+-independent kinases such as integrin-linked kinase, Rho-associated kinase, and zipper-interacting protein kinase may also phosphorylate smooth muscle RLC during sustained contractions (6, 13, 29, 35). Additional regulation through thin-filament or focal adhesion proteins may participate, but the physiological role of these is unclear (7, 19, 27, 32). The loss-of-function evidence from our previous reports suggests that Ca2+/calmodulin-dependent MLCK phosphorylation of RLC is critical for gut and airway smooth muscle contraction (11, 40), and we here investigate its importance for VSM function.

Depolarization of the VSM cell membrane activates voltage-gated Ca2+-channels, resulting in Ca2+ influx; agonist stimulation may activate G protein-coupled receptors (GPCR), leading to inositol 1,4,5-trisphosphate (IP3) formation with subsequent Ca2+ release from the sarcoplasmic reticulum (SR) and Ca2+ entry across plasma membrane (12, 18, 31). The increase in cytosolic Ca2+ leads to MLCK activation and RLC phosphorylation (16, 17). Activation of GPCRs may also enhance RLC phosphorylation through Ca2+-sensitization mechanisms. In a typical smooth muscle contraction, stimuli induce an initial, robust contraction along with a subsequent, sustained contraction. In contrast with phasic smooth muscle, tonic smooth muscle displays a prolonged sustained contraction.
prevailing explanation for this two-phase contraction is that Ca^{2+}-dependent MLCK initiates robust contraction by phosphorylating myosin RLC and that integrating signals produce sustained tension through Ca^{2+}-independent inhibition of MLCP activity with additional activity of Ca^{2+}-independent kinases (6, 13, 29, 33–35). To elucidate the role of VSM contractile regulation and its involvement in blood pressure, we focused on investigating the functional roles of Ca^{2+}-dependent MLCK in VSM in vivo. We analyzed mice with tissue-specific attenuation of MLCK and revealed the importance of MLCK in VSM contraction. We also investigated the role of MLCK in the maintenance of basal blood pressure and salt-induced hypertension. We thus provide an animal model of impaired vascular contractility, which might be used to further investigate signaling mechanisms for blood pressure regulation.

MATERIALS AND METHODS

Chemicals and antibodies. Norepinephrine (NE), phenylephrine (PE), ANG II, and endothelin-1 (ET-1) were purchased from Sigma (St. Louis, MO). Antibodies used in this study were to MLCK (K36; Sigma), Rho-associated coiled-coil-forming protein kinase II (ROCK-II; Santa Cruz Biotechnology, Santa Cruz, CA), regulatory light chain (RLC) (14), smooth muscle myosin heavy chain (SM-MHC; Abcam, Cambridge, MA), α-smooth muscle actin (α-SMA RB-9010; Thermo Scientific, Fremont, CA), myosin phosphatase targeting subunit of the RLC phosphatase (MYPT1; Upstate, Billerica, MA), and phospho-MYPT1[Thr-696] (Upstate), phospho-MYPT1[Thr-850] (Upstate), integrin-linked kinase (ILK; Sigma), and β-actin (Sigma).

Smooth muscle-specific Mlck knockout mice. The generation of tamoxifen inducible smooth muscle-specific Mlck knockout mice (MLCK^{smKO}) has been described previously (11). Mlck^{flox/+}; SM-CreERT2 mice received 5-day consecutive tamoxifen injections for generating MLCK^{smKO} mice. Mlck^{flox/+}; SM-CreERT2 mice with tamoxifen injection were used as controls (CTR). All animal experiments were approved by the Animal Care and Use Committee of Model Animal Research Center of Nanjing University.

Blood pressure recordings. Blood pressure was measured by using a validated pulse-based tail-cuff method as described (37). Mice were fixed in restrainers and placed in a warming chamber (32°C). Each tail was placed into a tail cuff, and pulsations were detected by a pulse sensor (ALC-NIBP system; Shanghai Alcott Biotech). The pressure at the point of disappearance of pulses upon inflation of the occlusion cuff was recorded to estimate blood pressure. All mice (10 wk old) received 5 days of training before data collection. Measurements were performed at 10 to 11 AM. Daily measurements started on the first day of tamoxifen treatment. To measure the blood pressure in response to vasoconstrictors, each vasoconstrictor was dissolved in 200 μl of physiological saline buffer and injected into the tail vein in a bolus dose. The measurements of blood pressure were taken for 15 min each and dried to remove residual ether. The protein was removed. Mice were allowed to recover for at least 1 wk. DOCA pellets (50 mg DOCA, 21-day release time; Innovative Research of America, Sarasota, FL) were implanted subcutaneously on the lateral side of the neck in anesthetized mice. Mice received 1% NaCl in the drinking water after DOCA administration. Blood pressure was measured by the tail-cuff method.

Histology and immunohistochemistry. Aortic, femoral, coronary, and mesenteric arteries were fixed with 4% formalin, embedded in paraffin, and transversely sectioned at a thickness of 6 μm. Tissue sections were stained with hematoxylin and eosin to examine artery histology. For immunohistochemistry, cryosections (10 μm) were fixed in cold acetone, and the nonspecific binding of primary antibody was blocked with 5% bovine serum albumin in phosphate-buffered saline. After washing with the same buffer, overnight incubation with antibodies to MLCK or α-smooth muscle actin was performed at 4°C. FITC-labeled rabbit-anti-mouse IgG (Sigma) or RITC-labeled goat-anti-mouse IgG (Pierce, Rockford, IL) was used individually as secondary antibodies. Images of immunofluorescence were acquired with a confocal microscope (Leica, TCS-SP2, Wetzlar, Germany).

Western blot. Western blot analyses were performed for measurements of MLCK and other proteins (11). Briefly, tissue samples were collected and frozen quickly in 10% trichloroacetic acid and 10 mM dithiothreitol in acetone precooled to slush at −80°C. After thorough homogenization, the sample pellet was washed three times with ether for 5 min each and dried to remove residual ether. The protein was dissolved completely in 8 M urea solution. Protein concentration was measured with bicinechonic acid (BCA) protein assay reagent. Aliquots were added to SDS sample buffer and boiled briefly. Equal amounts of protein were loaded for 6% SDS-PAGE followed by protein transfer to a nitrocellulose membrane. The membrane was probed with primary antibody and appropriate secondary antibody sequentially and then visualized by incubation in Super Signal West Dura substrate (Pierce) before exposure to film.

Measurement of myosin RLC phosphorylation. Urea/glycerol-PAGE was used for measurement of RLC phosphorylation where the nonphosphorylated RLC is separated from the monophosphorylated RLC (14). RLC protein was visualized by Western blot assay with an antibody to RLC. The percentage of phosphorylated RLC relative to total RLC was quantified with a Jieda 801 Image Analysis system 3.3.2 (JEDA Science-Technology Development, Nanjing, China).
RESULTS

Attenuation of MLCK and phenotypic characterization of VSMs. MLCK was disrupted specifically in smooth muscle cells of MLCKSMKO (MLckfloxflox, SM-CreERT2) mice by using a tamoxifen-inducible Cre-loxP approach (11). To examine the knockout efficiency of MLCK in arterial smooth muscle, we focused on mesenteric arteries. Western blot assays showed a variable reduction of MLCK protein in mesenteric arteries from different MLCKSMKO mice (Fig. 1A). The number of MLCK-deficient arteries, which expressed <5, 5–10, and 10% of residual MLCK protein, compared with CTR, is in the ratio of 5:3:2. The average value of retained MLCK is 6.5 ± 1.6% in mesenteric arteries from MLCKSMKO mice (N = 10; Fig. 1D). Immunohistochemical staining with anti-MLCK antibody also showed a marked reduction of MLCK protein. Figure 1B shows representative images for staining of MLCK in mesenteric arteries from CTR and MLCKSMKO mice where arterial MLCK contents are less than 5% of CTR. The smooth muscle layers of the artery wall showed significantly reduced MLCK expression, whereas the adventitia and intima showed positive staining (Fig. 1B).

To assess possible compensatory effects on myosin signaling, we evaluated the expression of ILK, ROCK II, MYPT1, and SM-MHC by Western blotting mesenteric arteries from CTR and MLCKSMKO mice. Results showed no significant differences in amounts of these proteins (Fig. 1, C and D), similar to observations from gut and airway smooth muscles (11, 40). Phosphorylation of MYPT1 in response to NE was robust in arteries from both CTR and MLCKSMKO mice (Supplemental Fig. 1, A–C). To test whether RhoA/Rho kinase-mediated inhibition of MLCP was impaired in MLCK-attenuated arteries, Rho kinase-specific inhibitor Y27632 was cumulatively added to mesenteric arteries contracted by KCl. As shown in Supplemental Fig. S1, D–F, 10 μM of Y27632 almost completely inhibited the sustained contraction in MLCK-attenuated arteries. Interestingly, arteries from these mice were even more sensitive to Y27632 compared with those from control mice. These results suggest a greater dependence on the RhoA/Rho kinase system when MLCK expression is attenuated.

No striking differences were observed in the morphology of arteries from CTR and MLCKSMKO mice at day 16 after tamoxifen injection except for decreased perivascular fat content in MLCKSMKO mice (Fig. 2 and 3). Whereas the gross morphology of the mesenteric arterial bed in vivo appeared similar, mesenteric veins from MLCKSMKO mice exhibited an enlarged lumen compared with CTR mice (Fig. 2, A–D). Diameter measurements of the secondary branches of mesenteric veins showed a significant increase in MLCKSMKO mice (299 ± 10 μM CTR and 434 ± 27 μM MLCKSMKO, P < 0.01; Fig. 2E). The dilation of mesenteric veins may result from contractile dysfunction associated with MLCK attenuation in venous smooth muscle cells. In fixed tissues, there were no gross differences in the appearance of several arteries including aorta, femoral artery, coronary artery, and mesenteric artery (Fig. 3). In a functional assessment of arterial diameter and compliance, the internal circumferences of relaxed, MLCK-deficient mesenteric arteries were compared with controls in a procedure that estimates the circumference corresponding to a
transmural pressure of 100 mmHg (IC100, automatically calculated by myograph normalization procedure) (28). IC100 values were comparable between MLCKSMKO and CTR vessels (321 ± 12 μm vs. 320 ± 18 μm, P > 0.05; Fig. 2F). This result suggests that the mesenteric arteries in MLCKSMKO mice have normal elastic properties.

Impaired VSM contraction in MLCKSMKO mice. Contractile properties of MLCK-deficient VSM were measured on segments of mesenteric artery by myography. With depolarization by 124 mM KCl, both the transient and sustained phases of force in mesenteric arteries prepared from MLCKSMKO mice were significantly smaller than that from control, but the contraction showed a typical force pattern (transient: CTR, 3.0 ± 0.2 mN; MLCKSMKO, 1.2 ± 0.1 mN, P < 0.01; sustained: CTR, 2.8 ± 0.3 mN; MLCKSMKO, 0.9 ± 0.1 mN, P < 0.01). Dose-response curves also showed reduced ability of KCl to induce contraction in MLCK-attenuated arteries (Fig. 4, A and B). The reduced RLC phosphorylation in response to KCl was consistent with the prominent attenuation of force development by VSM from MLCKSMKO mice. RLC phosphorylation values with NE stimulation were also significantly reduced in MLCKSMKO artery (Fig. 4, E and F). Thus MLCK appears to be the predominant kinase for RLC phosphorylation in VSM contractions.

Attenuation of MLCK in smooth muscle causes decreased physiological blood pressure and response to vasoconstrictors. We made daily measurements of systolic blood pressure (SBP) in Mlckfloxflox; SM-CreERT2 mice during and after tamoxifen transmural pressure of 100 mmHg (IC100, automatically calculated by myograph normalization procedure) (28). IC100 values were comparable between MLCKSMKO and CTR vessels (321 ± 12 μm vs. 320 ± 18 μm, P > 0.05; Fig. 2F). This result suggests that the mesenteric arteries in MLCKSMKO mice have normal elastic properties.

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Diminished myosin light chain phosphorylation of MLCKSMKO VSMs. We measured RLC phosphorylation in mesenteric arteries from MLCKSMKO mice in response to KCl-induced depolarization. In CTR arteries, RLC phosphorylation increased to 72 ± 8% by 10 s after KCl stimulation and was then maintained at a high level for more than 1 min. However, MLCKSMKO artery segments displayed less than 20% of RLC phosphorylation compared with CTR segment at all time points after stimulation (Fig. 4, B and C). The reduced RLC phosphorylation in response to KCl was consistent with the prominent attenuation of force development by VSM from MLCKSMKO mice. RLC phosphorylation values with NE stimulation were also significantly reduced in MLCKSMKO artery (Fig. 4, E and F). Thus MLCK appears to be the predominant kinase for RLC phosphorylation in VSM contractions.

Attenuation of MLCK in smooth muscle causes decreased physiological blood pressure and response to vasoconstrictors. We made daily measurements of systolic blood pressure (SBP) in Mlckfloxflox; SM-CreERT2 mice during and after tamoxifen
treatment. SBP of MLCKSMKO mice gradually decreased beginning 5–7 days after the last injection (Fig. 5A). By 16 days after starting tamoxifen injections, SBP decreased from 114/110 to 85/110 mmHg (P < 0.05; Fig. 5A). Thus attenuation of MLCK leads to a lower basal blood pressure.

To determine the response to agonists in vivo, mice were injected intravenously with various vasopressors, and the responses were expressed as the maximum increase in blood pressure (Fig. 5B). When compared with CTR, the response to NE was significantly less in MLCKSMKO mice (change of SBP: CTR, 67 ± 6 mmHg; MLCKSMKO, 20 ± 6 mmHg, P < 0.05). The responses to PE and ET-1 were also decreased in MLCKSMKO mice (Fig. 5B). This result shows that MLCKSMKO mice have significantly lower vascular responses to agonists, suggesting a general impairment of GPCR-mediated signaling to elevate blood pressure.

To assess possible cardiac and renal contributions to blood pressure regulation in MLCKSMKO mice, we measured cardiac function by echocardiography and renin, angiotensin, and aldosterone concentrations by ELISA. Measurements were made on mice at 15 days after initiating tamoxifen treatment. Two-dimensional-color Doppler ultrasound imaging revealed comparable cardiac functional parameters in CTR and MLCKSMKO mice including fractional shortening, ejection fraction, and ventricular volume (Table 1). Biochemical measurements of renin, ANG II, and aldosterone in peripheral blood were also comparable in CTR and MLCKSMKO mice (Table 2). Thus the MLCKSMKO and CTR mice appear to have comparable cardiac function and renal endocrine performance.

Attenuation of MLCK attenuates DOCA-salt-induced hypertension. GPCR-mediated signaling pathways differentially regulate physiological and pathological blood pressure through MLCK activation and/or MLCP inactivation, coordinated with other signaling modules (38). Dietary salt intake and a tendency toward salt retention are important in the pathogenesis of hypertension (1, 24). We here analyzed the effect of MLCK knockout on elevated blood pressure induced by DOCA and NaCl to assess the role of MLCK in hypertension. We selected the DOCA-salt model since this animal model is a well-established, clinically relevant model of systemic hypertension. In CTR mice, DOCA-salt treatment produced a robust increase in blood pressure within 5 days that was subsequently maintained at a high level (SBP: elevated from 103/100 to 143/132 mmHg; Fig. 6). A similar effect was observed in Mlckflox/flox; SM-CreERT2 mice without tamoxifen induction (SBP: from 98 ± 4 mmHg to 132 ± 2 mmHg). These results...
show salt-induction of hypertension. The elevated blood pressure of CTR mice was transiently and slightly reduced during the period of tamoxifen injections, but then returned to a high level (−140 mmHg; Fig. 6). Mlk^{flox/flox}; SM-CreERT2 mice displayed a progressive decrease in blood pressure after tamoxifen treatment, reaching values below normotensive levels (SBP decreased from 132 to 87 mmHg). Thus attenuation of MLCK expression led to a failure to maintain DOCA-salt-induced hypertension.

**DISCUSSION**

In response to vasoconstrictors, VSM produces robust and prolonged sustained force (30). The established regulatory scheme for this process involves initiation of robust contraction through RLC phosphorylation by Ca\(^{2+}\)-dependent MLCK and maintenance of sustained contraction through Ca\(^{2+}\)-sensitization by G_{q12-G_{13}}Rho/Rho kinase-mediated inhibition of MLCP (15, 22). Additionally, Ca\(^{2+}\)-independent kinases and thin filament-based regulation have been suggested to play a role in regulation of contraction (6, 7, 13, 19, 27, 29, 32, 35). We found in this study that profound reduction of Ca\(^{2+}\)-dependent MLCK impaired both the robust contraction and force maintenance in response to depolarization as well as vasoconstrictors. In addition, RhoA/ROCK phosphorylation of MYPT1 was not affected in arteries from Mlk^{smko} mice. Thus MLCK appears to be required both for initiation and maintenance of arterial contraction. RLC phosphorylation was also accordingly inhibited after MLCK attenuation, indicating that MLCK was the predominant kinase for RLC phosphorylation in VSM. Similar to results with intestinal and airway smooth muscles from Mlk^{smko} mice, mesenteric arteries retained a small contractile response to both depolarization and agonist that was associated with RLC phosphorylation (11, 40). Although the origins of the contraction are not defined, we consider the possibility that residual MLCK is available in an amount sufficient to phosphorylate a small fraction of RLC leading to initiation of contraction, which would be sustained by inhibition of MLCP. Whereas we previously showed that

Table 1. Echocardiographic assessment of CTR and Mlk^{smko} mice

<table>
<thead>
<tr>
<th>Parameter</th>
<th>CTR</th>
<th>Mlk^{smko}</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>LV</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fractional shortening, %</td>
<td>32.6 ± 1.6</td>
<td>33.5 ± 1.2</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Ejection fraction, %</td>
<td>61.4 ± 2.0</td>
<td>63.4 ± 1.8</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Mass, mg</td>
<td>73.2 ± 4.8</td>
<td>67.2 ± 7.1</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>LV volume, μl</td>
<td>55.8 ± 4.7</td>
<td>46.3 ± 5.3</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Diastole</td>
<td>21.5 ± 2.4</td>
<td>16.9 ± 2.2</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Systole</td>
<td>0.74 ± 0.05</td>
<td>0.75 ± 0.06</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>LV inner distance, mm</td>
<td>0.83 ± 0.06</td>
<td>0.83 ± 0.06</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Diastole</td>
<td>3.61 ± 0.10</td>
<td>3.35 ± 0.19</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Systole</td>
<td>2.43 ± 0.10</td>
<td>2.21 ± 0.12</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>LV posterior wall, mm</td>
<td>0.79 ± 0.03</td>
<td>0.76 ± 0.05</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Diastole</td>
<td>1.04 ± 0.05</td>
<td>1.00 ± 0.06</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Systole</td>
<td>386 ± 10</td>
<td>384 ± 20</td>
<td>&gt;0.05</td>
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</tbody>
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Values are means ± SE; N = 12 for control (CTR) mice and N = 8 for smooth muscle-specific MLCK knockout mice (Mlk^{smko}) mice. LV, left ventricular. Measurements were made on mice at 15 days after initiating tamoxifen treatment.

Table 2. Plasma renin, ANG II, and aldosterone in CTR and Mlk^{smko} mice

<table>
<thead>
<tr>
<th>Parameter, pg/ml</th>
<th>CTR</th>
<th>Mlk^{smko}</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Renin</td>
<td>21.6 ± 3.2</td>
<td>18.7 ± 2.5</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>ANG II</td>
<td>212 ± 20</td>
<td>265 ± 40</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Aldosterone</td>
<td>753 ± 55</td>
<td>637 ± 95</td>
<td>&gt;0.05</td>
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Values are means ± SE; N = 9–14. Samples were collected at 15 days after initiating tamoxifen treatment.
the fractional activation of a biosensor MLCK is only 0.25 for maximal agonist-induced contraction, the 6.5% remaining kinase in the MLCKSMKO vessels may be sufficient for a limited contraction with enhanced MLCP inhibition (14). The apparent greater sensitivity to the inhibitory effects of the ROCK inhibitor in arteries from MLCKSMKO mice supports this idea. He et al. (11) also showed that the limited residual contraction was calcium dependent, ruling out a role for calcium-independent kinases. Further experiments are required to define the properties of the residual contraction in smooth muscles from these mice.

Basal blood pressure is dependent on multiple factors, including circulating volume, cardiac output, renal function, and hormone-, paracrine-, or myogenic-regulated vascular tone. Our results showed that vasorelaxation mediated by attenuation of MLCK was sufficient to reduce basal blood pressure. The SBP of MLCKSMKO mice fell to 85 mmHg at day 16 after initiating tamoxifen treatment, having begun to drop rapidly over preceding days. MLCKSMKO mice succumb at initiating tamoxifen treatment, having begun to drop rapidly over preceding days. MLCKSMKO mice succumb at day 17 from a general failure of smooth muscle contraction that includes paralytic ileus (11). A SBP of 85 mmHg would not be expected to cause lethality (26), so it is unlikely that the animals succumb from severe hypertension.

The importance of the kidney for the pathogenesis of hypertension is well established where the renin-angiotensin system plays a major role (8, 21). Molecular genetic studies have identified mutations in several genes linked to hypertension or hypotension, and these generally act in the physiological pathway that alters net renal salt reabsorption (21). However, it has also been shown that, during the development of hypertension, MLCK expression may be upregulated in blood vessels, implying a functional role for MLCK in progression of hypertension (9). In the present study, we found that attenuation of MLCK was able to abolish salt-induced hypertension. This effect does not appear to be due to primary cardiac and renal dysfunction or secondary compensatory effects after MLCK attenuation, because the cardiac functional parameters and serum renin/ANG II/aldosterone levels in MLCKSMKO mice are not obviously altered. Because MLCK is a common module of the signaling pathway regulating smooth muscle contraction, the conclusion that MLCK supports salt-induced hypertension might be considered for other kinds of hypertension. Additionally, our results emphasize the importance of vascular contractility in hypertension.

Phenotypic comparison among mice with smooth muscle-specific knockouts of Cav1.2, GPCR, and MLCK sheds light on the role of VSM signaling cascades in blood pressure regulation. Deletion of Cav1.2, an essential L-type calcium channel for influx of calcium and subsequent MLCK activation during depolarization-induced contraction, causes reductions in VSM contraction and basal blood pressure similar to the phenotype of MLCK-deficient mice (26). Thus the Ca1.2/MLCK pathway in VSM appears necessary for regulation of basal blood pressure. Smooth muscle-specific knockout of G_{qG}G_{a11} in mice impairs basal blood pressure maintenance and development of salt-induced hypertension, whereas deletion of G_{q12}G_{a13} inhibits salt-induced hypertension without affecting basal blood pressure (38, 39). In this study, we found that attenuation of MLCK not only impaired the maintenance of physiological blood pressure but also abolished salt-induced hypertension. Collectively, G_{qG}G_{a11}/MLCK signaling appears essential for physiological maintenance of blood pressure, whereas the G_{q12}G_{a13}/MLCP signaling pathway may be required for salt-induced hypertension in addition to MLCK activation.

In summary, we established an animal model of impaired vascular contractility, demonstrating the essential role of MLCK in VSM contraction and blood pressure regulation in vivo. Because MLCK is central to RLC phosphorylation and myosin cross bridge cycling, its absence impairs contractile responses to a wide variety of agonists with markedly different transduction pathways.

ACKNOWLEDGMENTS

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GRANTS

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

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