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Metformin-induced AMP-activated protein kinase activation regulates phenylephrine-mediated contraction of rat aorta

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ABSTRACT

The aim of the present study is to determine the effects and molecular mechanisms by which activation of LKB1–AMP-activated protein kinase (AMPK) by metformin regulates vascular smooth muscle contraction. The essential ability of vascular smooth muscle cells (VSMCs) to contract and relax in response to an elevation and reduction in intravascular pressure is necessary for appropriate blood flow regulation. Thus, vessel contraction is a critical mechanism for systemic blood flow regulation. In cultured rat VSMCs, AMPK activation through LKB1 by metformin-inhibited phenylephrine-mediated myosin light chain kinase (MLCK) and myosin light chain phosphorylation (p-MLC). Conversely, inhibition of AMPK and LKB1 reversed phenylephrine-induced MLCK and p-MLC phosphorylation. Measurement of the tension trace in rat aortic rings also showed that the effect of AMPK activation by metformin decreased phenylephrine-induced contraction. Metformin inhibited PE-induced p-MLC and α-smooth muscle actin co-localization. Our results suggest that activation of AMPK by LKB1 decreases VSMC contraction by inhibiting MLCK and p-MLC, indicating that induction by the AMPK–LKB1 pathway may be a new therapeutic target to lower high blood pressure.

1. Introduction

AMP-activated protein kinase (AMPK) is relevant as a latent regulator of vascular function [1]. Although AMPK is recognized as a cellular energy sensor, identifying its ability to be stimulated by chemical mediators of vasomotor function has suggested a critical role for AMPK in the regulation of vascular control [2]. Of particular importance, the LKB1–AMPK signaling pathway has not been demonstrated in vascular smooth muscle. Vascular smooth muscle in large vessels such as the aorta maintains contractile force with no measurable changes in cellular ATP [3]. Lower metabolic rates during contraction make it possible for metabolism to provide the energy needs of vascular smooth muscle [4]. However, it has not yet been reported whether AMPK plays a particular role regulating metabolic pathways in smooth muscle during contraction. Many vasomotor tone studies and biochemical experiments have shown that activated endothelial AMPK increases phosphorylation and activation of endothelial nitric oxide synthase [5,6]. Contraction modulates gene expression and serine/threonine kinase activities and helps cause adaptive changes in skeletal muscle [7].

AMPK is a serine/threonine protein kinase activated by pathological stimuli, such as oxidative damage, and by physiological stimuli, such as exercise and muscle contraction [8]. Of AMPK upstream kinases, cytosolic calcium-dependent AMPK not only controls muscle contraction and relaxation but also serves as a second messenger to trigger pathways that regulate muscle function and energy metabolism [9]. In response to stimuli, AMPK is activated via phosphorylation by upstream kinases, LKB1, and calcium/calmodulin-dependent protein kinase (CaMKK). The tumor suppressor protein LKB1 activates AMPK in several cell types and CaMKK also phosphorylates AMPK Thr172 [10,11].

Smooth muscle contraction is based on the fundamental concept that myosin regulatory light chain phosphorylation, which is a function of the dynamic balance between the opposing activities of myosin light chain kinases (MLCK) and myosin light chain phosphorylation (p-MLC) [12]. MLCK and p-MLC initiate smooth muscle contraction also allow non-muscle myosin to interact with actin, inducing changes in the actin cytoskeleton critical for the regulation of cell polarity [13]. AMPK directly phosphorylates the regulatory Ser19 smooth muscle myosin light chain kinase (smMLCK) site of MLC *in vitro* and *in vivo* in mammals, and AMPK-null *Drosophila* mutants have severe abnormalities in cell structure similar to the LKB1 knockout phenotype [14]. Activated MLCK then phosphorylates the regulatory myosin light chains, triggering cross-bridge cycling and contraction. Here, we show that MLCK is a substrate of AMPK. Ca^{2+}/calmodulin-dependent activation of MLCK induces phosphorylation of myosin light chain, which initiates vascular smooth muscle contraction [15]. The ability of

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resistant arteries to react to vascular pressure to control blood flow can be traced to cellular mechanisms inherent to vascular smooth muscle cells (VSMCs) in the arterial wall [16]. This response is critically important for the development of resting vessel tone, on which other control mechanisms exert vasodilation and vasoconstriction effects. However, it has not yet been reported whether the AMPK signaling pathway is particularly related to the vasodilatory actions in VSMCs. In the present study, we investigated the possible involvement of the LKB1–AMPK pathway in this intracellular signaling pathway. The findings suggest that LKB1–AMPK pathway attenuates contraction by inactivating MLCK and phosphorylating MLC.

2. Materials and methods

2.1. Reagents and antibodies

MLCK and a monoclonal antibody against β-actin was purchased from Sigma (St. Louis, MO, USA). Antibodies specifically recognizing AMPK, p-AMPK, LKB1, and p-LKB1 were obtained from Cell Signaling Technology, Inc. (Beverly, MA, USA). p-MLC was obtained from Cell Signaling Technology, Inc. and Abcam Inc. (Cambridge, MA, USA). AMPK siRNA, LKB1 siRNA, and control siRNA were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). 1,1-Dimethylbiguanide hydrochloride (metformin) was purchased from Sigma–Aldrich. L-Phenylephrine (PE) was acquired from MP Biomedical, Inc. (Solon, OH, USA). Compound C, a AMPK inhibitor, was provided by Calbiochem (La Jolla, CA, USA).

2.2. Measurement of tension response in aortic rings

Male Sprague–Dawley rats (8 weeks) were euthanized by rapidly removing the aorta. The thoracic aorta was excised, 2-mm aortic rings were immersed in Krebs-bicarbonate buffer (117 mM NaCl, 4.8 mM KCl, 1.2 mM $MgSO_4$, 25 mM $NaHCO_3$, 1.2 mM KH_2PO_4, 5.7 mM glucose, 2.5 mM $CaCl_2$), and carefully cleaned of all fat and connective tissue. In our experiments, the endothelium was removed by inserting a titanium wire through the vessel lumen. The ring was mounted onto a vascular myography apparatus (Biopac System, Inc., Goleta, CA, USA) where it was immersed in 37 °C Krebs-bicarbonate buffer continuously aerated with 95% O_2 and 5% CO_2. Muscle tone was measured using an isometric force transducer. The artery segment was incubated for 30 min in Krebs solution and thereafter stimulated with compound C and PE.

2.3. Western blot analysis

Whole cell extracts were prepared by lysing the cells in Pro-Prep Protein Extract buffer. The protein concentration was quantified with protein assay reagent from Bio-Rad (Hercules, CA, USA). Equal amounts of protein were mixed with Laemmli Sample Buffer (Bio-Rad) and heated for 7 min at 100 °C before loading. Total protein samples (40 μg) were subjected to 10% SDS–polyacrylamide gel electrophoresis for 1 h 30 min at 100–120 V. The separated proteins were electrophoretically transferred onto a PVDF membrane for 1 h 20 min at 100 V using a SD Semi-dry Transfer Cell. The membranes were blocked with 5% non-fat milk in PBS buffer containing 0.05% Tween20 (PBST) for 2 h at room temperature. The membranes were then incubated with primary antibodies at a dilution of 1:1000 overnight at 4 °C in PBST. The membranes were then washed with four changes of wash buffer (0.05% Tween20 in PBS) and incubated for 1 h at room temperature in PBS containing anti-rabbit (Stressgen, Ann Arbor, MI, USA) or anti-mouse IgG (Santa Cruz Biotechnology) antibodies. Finally, after three more rinses with wash buffer, the membranes were exposed to enhanced chemiluminescent (ECL) and ECL Plus Western blot analysis detection reagents.

2.4. RNA isolation and real-time polymerase chain reaction (PCR) analysis

Total RNA was isolated from cultures using Trizol reagent (Invitrogen, Carlsbad, CA, USA) and reverse transcription was performed using SuperScript III reverse transcriptase (Invitrogen). We also determined MLCK mRNA levels in VSMCs using real-time PCR. The primers for the PCR analysis were synthesized at Macrogen (Daejeon, Korea). The primers were as follows: MLCK, forward (F) 5′-AATGGTGTTGCTGGAGATCGAGGT-3′ and reverse (R) 5′-GCTGGA TCAAATTGCGGTGGTTCA-3′. Real-time PCR was performed using the Quantitect SYBR Green kit (Applied Biosystems, Foster City, CA, USA) according to the manufacturer's instructions. Reaction volume was 20 μl, and annealing temperature was 57 °C. Fluorescence was detected using an ABI Prism 7700 Detection System. The ratios of target gene to GAPDH were calculated.

2.5. Immunofluorescence analysis

VSMCs were seeded on coverslips in 35-mm dishes, fixed in 4% formaldehyde, and permeabilized with 0.2% Triton X-100. The p-MLC primary antibody was used at 1:50 (Cell Signaling Technology) and incubated with cells overnight at 4 °C. Rabbit FITC secondary antibody was used at 1:100 (Molecular Probes, Eugene, OR, USA) and incubated with cells for 1 h at room temperature. Fixed and immunofluorescently stained cells were imaged with a Leica confocal microscope (Bannockburn, IL, USA).

2.6. Flow cytometry

Cells were washed and then maintained in complete medium. After detachment from dishes with 50 mM EDTA, the cells were centrifuged at 3000 rpm for 10 min and resuspended in PBS containing 2% bovine serum albumin. After labeling, cells were washed once in PBS, fixed in 4% paraformaldehyde, and analyzed on a flow cytometer. For each sample, 1000 cells were analyzed, and the results were expressed as geometric mean fluorescence.

2.7. Statistical analysis

All data are expressed as mean ± SEM. Differences between data sets were assessed by analysis of variance followed by Bonferroni's *t*-test. *P* values <0.05 were considered significant.

3. Results

3.1. Endothelium-independent vasomotor responses and inhibition of PE-induced MLCK and MLC phosphorylation by metformin

Metformin inhibited PE-induced contraction in aortic rings. As shown in Fig. 1A, metformin relaxed constricted aortic rings. The major molecular target of metformin is the AMPK cascade [17]. To investigate the effect of AMPK, we tested the effect of an AMPK inhibitor, compound C (10 μM), on PE-induced contraction. The inhibition of contraction induced by metformin was altered by pre-incubating the rings with compound C (Fig. 1A). The effects of metformin were due to LKB1–AMPK activation. We next examined whether AMPK activation could regulate PE-induced MLCK and p-MLC. To test this, we treated VSMCs with metformin and then measured MLCK and MLC phosphorylation by Western blot analysis. Treatment of VSMCs with PE (1 μM) significantly increased MLCK and p-MLC. As expected, pretreatment with metformin

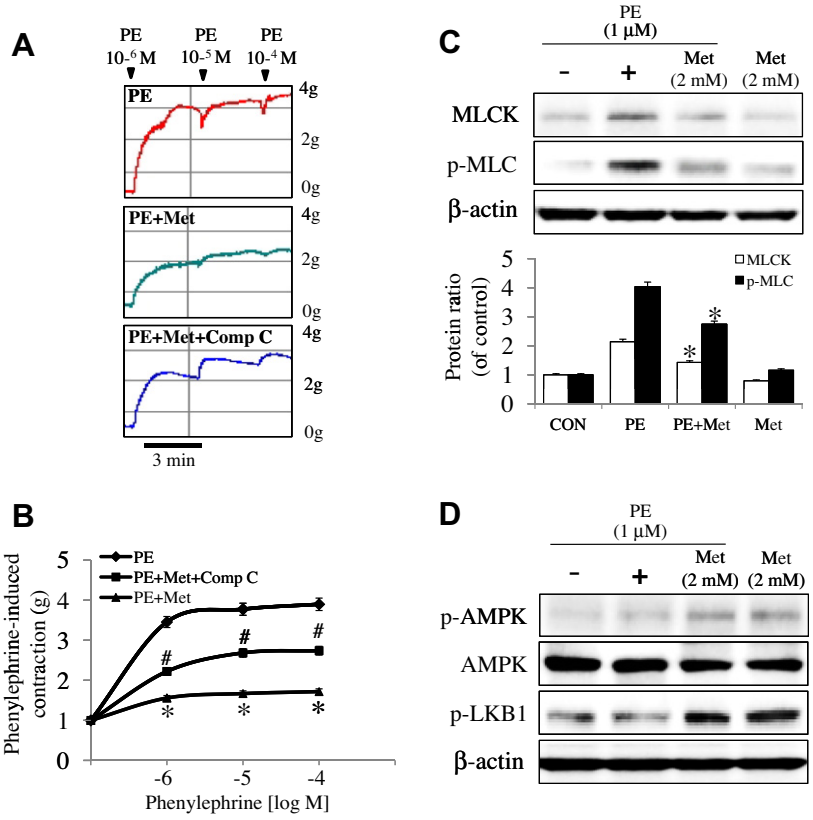

Fig. 1. Effect of metformin on the PE-induced contraction of rat aortic rings and the inhibitory effect of metformin on MLCK/MLC phosphorylation. Representative tension curves (A) and mean values (B) demonstrate the contraction-responses to increasing concentrations of PE after metformin (2 mM) and compound C (10 μM) treatment for each 30 min in isolated rat aortic rings. Muscle tone was measured using an isometric force transducer. The inhibitory effect of metformin on PE-induced contraction is shown without endothelium. Results are expressed as means ± SEM of five rat aortas. $^{*}P < 0.01$ vs. PE alone, $^{\#}P < 0.01$ vs. PE plus metformin. (C and D) show Western blot analysis of MLCK, p-MLC, p-AMPK, AMPK and p-LKB1. Activating LKB1–AMPK signaling through the metformin inhibited protein levels of PE-induced MLCK and p-MLC in VSMCs. VSMCs were incubated in the presence or absence of 1 μM PE for 30 min after pretreatment with 2 mM metformin for 1 h. Values are given as means ± SEM ($n = 3$). $^{*}P < 0.01$ vs. PE alone.

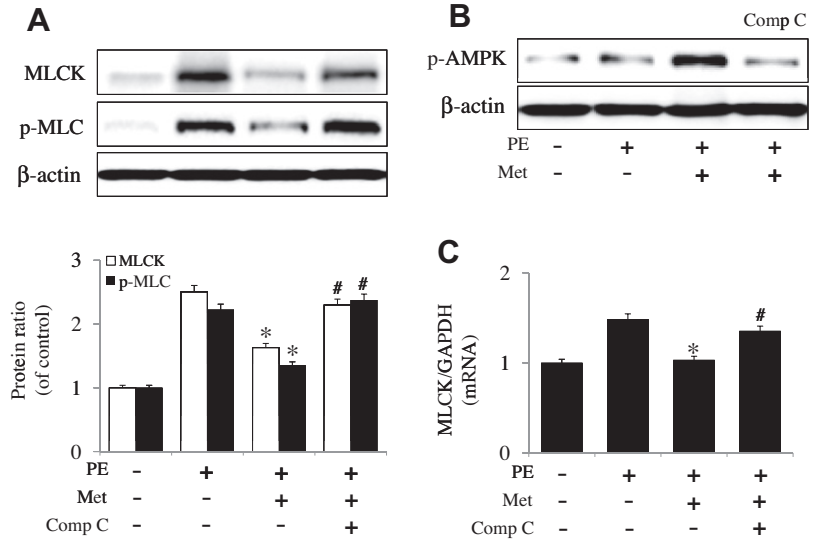

Fig. 2. Inhibitory effect of AMPK on protein levels of MLCK and p-MLC. (A and B) show the Western blot analysis of MLCK, p-MLC and p-AMPK in VSMCs pretreated with metformin (2 mM) or compound C (10 μM) for 1 h and then incubated with phenylephrine (PE) for 30 min. Inhibiting AMPK activation restored MLCK and p-MLC protein levels. Values are given as means ± SEM ($n = 3$). $^{*}P < 0.01$ vs. PE alone, $^{\#}P < 0.01$ vs. PE plus metformin. (C) MLCK mRNA levels in VSMCs were determined by q-PCR. Representative results from three independent experiments are shown. Values are given as means ± SEM ($n = 3$). $^{*}P < 0.001$ vs. PE alone, $^{\#}P < 0.01$ vs. PE plus metformin.

(2 mM) increased AMPK phosphorylation (Fig. 1D). However, metformin abolished protein levels of PE-induced MLCK and p-MLC (Fig. 1C) Vessel tension is determined by the status of the MLC in VSMCs [18]. Taken together, these results show that aortic contraction induced by PE was inhibited by LKB1–AMPK activation through metformin.

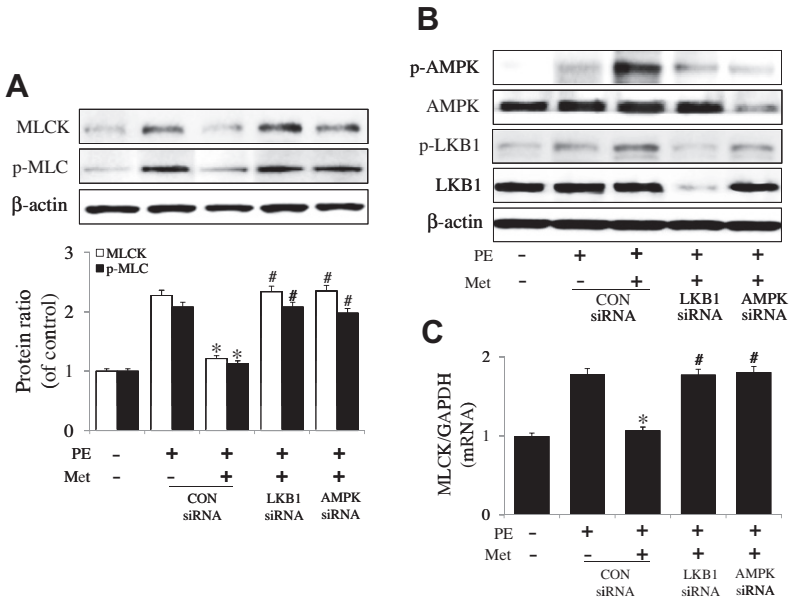

Fig. 3. LKB1–AMPK signaling pathway regulates expressions of MLCK and p-MLC. (A) Western blot analysis of MLCK and p-MLC in VSMCs were transfected with control siRNA, LKB1 siRNA, AMPK siRNA for 48 h and then incubated with metformin for 1 h and PE for 30 min. Values are given as means ± SEM ($n = 3$). $^{*}P < 0.01$ vs. PE plus control siRNA, $^{\#}P < 0.01$ vs. PE plus metformin in control siRNA. (B) VSMCs were subjected to Western blotting to determine the level of pAMPK, AMPK, p-LKB1, LKB1 proteins after transfections of control siRNA, LKB1 siRNA, and AMPK siRNA. (C) mRNA levels of MLCK in VSMCs were determined by using q-PCR. Representative results from three independent experiments were shown. Values are given as means ± SEM ($n = 3$). $^{*}P < 0.01$ vs. PE plus control siRNA, $^{\#}P < 0.01$ vs. PE plus metformin in control siRNA.

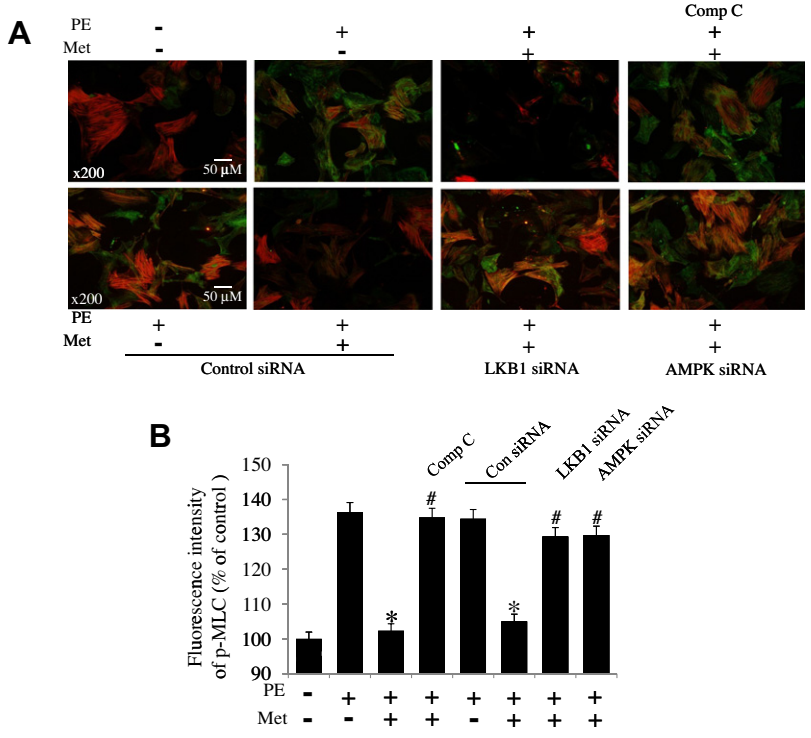

Fig. 4. Localization and flow cytometric analysis of p-MLC proteins regulated by metformin in VSMCs. (A) Cells are doubly stained with Alexa Fluor 546-labeled α-smooth muscle actin (red) and phosphorylated MLC (green). Yellow staining indicates the overlap of the two colors, where α-smooth muscle actin and p-MLC co-localize. All photographs are taken under confocal laser-scanning microscopy. VSMCs treated with compound C, LKB1 siRNA, and AMPK siRNA show more MLC phosphorylation. (B) Flow cytometric intensity of p-MLC protein in VSMCs. p-MLC level was detected with p-MLC antibody and the results were analyzed by FACS. Fluorescence intensity is geometric mean fluorescence. Each percentage is based on 1000 cells. $^{*}P < 0.05$ vs. PE alone, $^{\#}P < 0.01$ vs. PE plus metformin. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this paper.)

3.2. Inhibition of AMPK activation restores PE-induced MLCK and p-MLC

To investigate the potential effects of metformin, we next examined whether chemical inhibition of AMPK (compound C) affected PE-induced MLCK expression and MLC phosphorylation. In VSMCs treated with compound C, protein levels of PE-induced MLCK and MLC phosphorylation increased as in the PE alone treatment (Fig. 2A). Conversely, MLCK and MLC phosphorylation was decreased in VSMCs treated with metformin. After the PE and

metformin treatment, the MLCK mRNA level was also checked by real-time PCR analysis (Fig. 2C). Taken together, these data suggest that AMPK regulates PE-induced MLCK and MLC phosphorylation related to vessel smooth muscle contraction [19].

3.3. Inhibition of the LKB1–AMPK signaling pathway restores protein levels of PE-induced MLCK and MLC phosphorylation

Recently, LKB1 has been appreciated as a signaling protein [20]. LKB1 is the upstream kinase of AMPK and phosphorylates AMPK. AMPK activation by metformin is absent in LKB1-deficient cells [21]. To determine whether LKB1 plays a role in metformin-stimulated AMPK activation, we examined the effect of LKB1 siRNA and AMPK siRNA on VSMCs. Western blot analysis revealed that LKB1 siRNA and AMPK siRNA reversed MLCK and MLC phosphorylation (Fig. 3A). As shown in Fig. 3A and B, PE-induced MLCK and MLC phosphorylation was greater in cells transfected with LKB1–AMPK targeted siRNA than that in control siRNA-transfected cells and metformin-treated cells. The MLCK mRNA level was also checked by real-time PCR analysis (Fig. 3C). These results support that LKB1–AMPK activation through metformin regulates PE-induced MLCK and MLC phosphorylation in VSMCs.

3.4. Inhibiting LKB1–AMPK activation through metformin increases MLC phosphorylation

MLC phosphorylation is necessary to initiate contraction [22]. In smooth muscle cells, contractile power is generated by the interaction between myosin and actin, which is correlated with MLC phosphorylation levels [23]. To further test whether PE phosphorylates MLC, cultured cells were treated with compound C and transfected with LKB1 and AMPK siRNAs and then stained with p-MLC specific antibodies. The cells were doubly stained with Alexa Fluor 546-labeled α-smooth muscle actin (red) and Alexa Fluor 488-labeled MLC phosphorylation (green). Yellow staining indicates the overlap of the two colors, where p-MLC and α-smooth muscle actin co-localized. Immunofluorescence with the p-MLC antibodies revealed no changes in MLC phosphorylation in compound C-treated and siRNA transfected cells compared to PE alone cells. However, it should be noted that the p-MLC antibody did not recognize cells treated with PE plus metformin (Fig. 4A). In the next set of experiments, we examined this in flow cytometry studies (Fig. 4B). This analysis showed that the level of p-MLC decreased approximately 20% in cells incubated with metformin compared to that in the PE alone treatment. These results indicate that LKB1–AMPK signaling activated by metformin regulates MLC phosphorylation related to contraction for vascular tone.

4. Discussion

MLCK and MLC phosphorylation play a critical role controlling myosin contractility in smooth muscle [22]. The major finding of this study is that LKB1–AMPK activation by metformin suppressed VSMC contraction by inhibiting MLCK and MLC phosphorylation. Recent studies have found that AMPK-mediated cellular functions have protective effects that oppose many cardiovascular diseases [24,25]. In addition, AMPK activation by AICAR or resveratrol lowers blood pressure (BP) in obese rats [26,27]. Thus, inhibiting MLCK and MLC phosphorylation by LKB1–AMPK activation might be an important mechanism for lowering BP. However, these studies did not demonstrate a direct effect of LKB1–AMPK on BP. Vascular smooth muscle function is directly related to vascular tone, and abnormal VSMC relaxation or contraction induces hypertension in all animal models [28]. Importantly, metformin, one of the mostly widely used antidiabetic drugs, activates AMPK *in vivo* and lowers BP in various hypertensive animal models [29]. Interestingly, AMPK activation is necessary for the therapeutic actions of metformin in LKB1 knockout mice. In cultured bovine aortic endothelial cells, metformin dose-dependently activates AMPK by increasing the phosphorylated form and increasing the association of AMPK with LKB1, its upstream kinase [30]. Therefore, metformin is likely to increase AMPK activation by promoting phosphorylation. The interaction of AMPK and metformin produces cardioprotective effects. The cardioprotective benefits of metformin are mimicked by adding the AMPK agonist AICAR [31]. Therefore, we hypothesized that activating the LKB1–AMPK signaling pathway might inhibit vascular smooth muscle contraction. The aim of our study was to elucidate the mechanisms by which LKB1–AMPK activation attenuates PE-induced contraction of smooth muscle and the physiological functions associated with the LKB1–AMPK signaling pathway.

One of the most important findings of our study is that LKB1–AMPK activation by metformin regulates MLCK and MLC phosphorylation during the VSMC contraction process. This appears to occur indirectly through MLCK inactivation, which, in turn, inhibits MLC phosphorylation. This result is supported by several findings. First, activation of LKB1–AMPK by metformin attenuated PE-induced contraction (Fig. 1). Second, inhibiting the LKB1–AMPK pathway with a pharmacological inhibitor (compound C) and genetic inhibitors (LKB1 siRNA, AMPK siRNA) affected levels of MLCK and MLC phosphorylation (Figs. 2 and 3). Finally, metformin treatment abolished PE-induced MLCK and MLC phosphorylation. Conversely, the LKB1–AMPK inhibitors potentiated PE-induced activation of MLCK and MLC phosphorylation. The effects of metformin on vascular reactivity were markedly reduced by the LKB1–AMPK inhibitors. Taken together, our results confirm a direct inhibitory effect of LKB1–AMPK on VSMC contraction and the effects of metformin on vascular contraction result from the enhanced phosphorylation of LKB1–AMPK in aortic smooth muscle. The combination of these results will contribute to BP regulation in the rat.

Data from the present study demonstrate that the LKB1–AMPK pathway is regulated during smooth muscle contraction. Further studies to elucidate the mechanism of AMPK activation in smooth muscle and its mechanism of LKB1–AMPK activation in smooth muscle as well as its integration with other signaling pathways will be critical to understand the complex kinase pathways regulating smooth muscle contraction processes. Our results provide a rationale for the therapeutic use of metformin for vascular dysfunction in patients with hypertension.

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References

[1] D.G. Hardie, J.W. Scott, D.A. Pan, E.R. Hudson, Management of cellular energy by the AMP-activated protein kinase system, FEBS Lett. 546 (2003) 113–120.

[2] R.J. Ford, J.W. Rush, Endothelium-dependent vasorelaxation to the AMPK activator AICAR is enhanced in aorta from hypertensive rats and is NO and EDCF dependent, Am. J. Physiol. Heart Circ. Physiol. 300 (2011) 64–75.

[3] C.D. Hardin, R.W. Wiseman, M.J. Kushmerick, Tension responses of sheep aorta to simultaneous decreases in phosphocreatine, inorganic phosphate and ATP, J. Physiol. 458 (1992) 139–150.

[4] J.M. Krisanda, R.J. Paul, Phosphagen and metabolite content during contraction in porcine carotid artery, Am. J. Physiol. 244 (1983) 385–390.

[5] V.A. Morrow, F. Foufelle, J.M. Connell, J.R. Petrie, G.W. Gould, I.P. Salt, Direct activation of AMP-activated protein kinase stimulates nitric-oxide synthesis in human aortic endothelial cells, J. Biol. Chem. 278 (2003) 31629–31639.

[6] Z. Chen, I.C. Peng, W. Sun, M.I. Su, P.H. Hsu, Y. Fu, Y. Zhu, K. DeFea, S. Pan, M.D. Tsai, J.Y. Shyy, AMP-activated protein kinase functionally phosphorylates endothelial nitric oxide synthase Ser633, Circ. Res. 104 (2009) 496–505.
[7] R. Bassel-Duby, E.N. Olson, Signaling pathways in skeletal muscle remodeling, Annu. Rev. Biochem. 75 (2006) 19–37.
[8] J. Mu, J.T. Brozinick Jr., O. Valladares, M. Bucan, M.J. Birnbaum, A role for AMP-activated protein kinase in contraction- and hypoxia-regulated glucose transport in skeletal muscle, Mol. Cell 7 (2001) 1085–1094.
[9] E.R. Chin, Role of Ca^{2+}/calmodulin-dependent kinases in skeletal muscle plasticity, J. Appl. Physiol. 99 (2005) 414–423.
[10] A. Woods, S.R. Johnstone, K. Dickerson, F.C. Leiper, L.G. Fryer, D. Neumann, U. Schlattner, T. Wallimann, M. Carlson, D. Carling, LKB1 is the upstream kinase in the AMP-activated protein kinase cascade, Curr. Biol. 13 (2003) 2004–2008.
[11] A. Woods, K. Dickerson, R. Heath, S.P. Hong, M. Momcilovic, S.R. Johnstone, M. Carlson, D. Carling, Ca^{2+}/calmodulin-dependent protein kinase kinase-beta acts upstream of AMP-activated protein kinase in mammalian cells, Cell Metab. 2 (2005) 21–33.
[12] K. Hirano, Current topics in the regulatory mechanism underlying the Ca^{2+} sensitization of the contractile apparatus in vascular smooth muscle, J. Pharmacol. Sci. 104 (2007) 109–115.
[13] S.E. Thatcher, M.E. Fultz, H. Tanaka, H. Hagiwara, H.L. Zhang, Y. Zhang, K. Hayakawa, S. Yoshiyama, A. Nakamura, H.H. Wang, T. Katayama, M. Watanabe, Y. Lin, G.L. Wright, K. Kohama, Myosin light chain kinase/actin interaction in phorbol dibutyrate-stimulated smooth muscle cells, J. Pharmacol. Sci. 116 (2011) 116–127.
[14] J.H. Lee, H. Koh, M. Kim, Y. Kim, S.Y. Lee, R.E. Karess, S.H. Lee, M. Shong, J.M. Kim, J. Kim, J. Chung, Energy-dependent regulation of cell structure by AMP-activated protein kinase, Nature 447 (2007) 1017–1020.
[15] R.S. Adelstein, E. Eisenberg, Regulation and kinetics of the actin–myosin–ATP interaction, Annu. Rev. Biochem. 49 (1980) 921–956.
[16] M.J. Davis, M.A. Hill, Signaling mechanisms underlying the vascular myogenic response, Physiol. Rev. 79 (1999) 387–423.
[17] S.A. Hawley, A.E. Gadalla, G.S. Olsen, D.G. Hardie, The antidiabetic drug metformin activates the AMP-activated protein kinase cascade via an adenine nucleotide-independent mechanism, Diabetes 51 (2002) 2420–2425.
[18] A.P. Somlyo, A.V. Somlyo, Ca^{2+} sensitivity of smooth muscle and nonmuscle myosin II: modulated by G proteins, kinases, and myosin phosphatase, Physiol. Rev. 83 (2003) 1325–1358.
[19] S. Horman, N. Morel, D. Vertommen, N. Hussain, D. Neumann, C. Beauloye, N. El Najjar, C. Forcet, B. Viollet, M.P. Walsh, L. Hue, M.H. Rider, AMP-activated protein kinase phosphorylates and desensitizes smooth muscle myosin light chain kinase, J. Biol. Chem. 283 (2008) 18505–18512.
[20] D.R. Alessi, K. Sakamoto, J.R. Bayascas, LKB1-dependent signaling pathways, Annu. Rev. Biochem. 75 (2006) 137–163.
[21] S.A. Hawley, J. Boudeau, J.L. Reid, K.J. Mustard, L. Udd, T.P. Mäkelä, D.R. Alessi, D.G. Hardie, Complexes between the LKB1 tumor suppressor, STRAD alpha/beta and MO25 alpha/beta are upstream kinases in the AMP-activated protein kinase cascade, J. Biol. 2 (2003) 28.
[22] B.G. Allen, M.P. Walsh, The biochemical basis of the regulation of smooth-muscle contraction, Trends Biochem. Sci. 19 (1994) 362–368.
[23] D.J. Hartshorne, Biochemical basis for contraction of vascular smooth muscle, Chest 78 (1980) 140–149.
[24] Y. Dong, M. Zhang, S. Wang, B. Liang, Z. Zhao, C. Liu, M. Wu, H.C. Choi, T.J. Lyons, M.H. Zou, Activation of AMP-activated protein kinase inhibits oxidized LDL-triggered endoplasmic reticulum stress in vivo, Diabetes 59 (2010) 1386–1396.
[25] Y. Dong, M. Zhang, B. Liang, Z. Xie, Z. Zhao, S. Asfa, H.C. Choi, M.H. Zou, Reduction of AMP-activated protein kinase alpha2 increases endoplasmic reticulum stress and atherosclerosis in vivo, Circulation 121 (2010) 792–803.
[26] E.S. Buhl, N. Jessen, R. Pold, T. Ledet, A. Flyvbjerg, S.B. Pedersen, O. Pedersen, O. Schmitz, S. Lund, Long-term AICAR administration reduces metabolic disturbances and lowers blood pressure in rats displaying features of the insulin resistance syndrome, Diabetes 51 (2002) 2199–2206.
[27] L. Rivera, R. Morón, A. Zarzuelo, M. Galisteo, Long-term resveratrol administration reduces metabolic disturbances and lowers blood pressure in obese Zucker rats, Biochem. Pharmacol. 77 (2009) 1053–1063.
[28] S.K. Michael, H.K. Surks, Y. Wang, Y. Zhu, R. Blanton, M. Jamnongjit, M. Aronovitz, W. Baur, K. Ohtani, M.K. Wilkerson, A.D. Bonev, M.T. Nelson, R.H. Karas, M.E. Mendelsohn, High blood pressure arising from a defect in vascular function, Proc. Natl. Acad. Sci. USA 105 (2008) 6702–6707.
[29] A.K. Wong, J. Howie, J.R. Petrie, C.C. Lang, AMP-activated protein kinase pathway: a potential therapeutic target in cardiometabolic disease, Clin. Sci. 116 (2009) 607–620.
[30] M.H. Zou, S.S. Kirkpatrick, B.J. Davis, J.S. Nelson, W.G. Wiles 4th, U. Schlattner, D. Neumann, M. Brownlee, M.B. Freeman, M.H. Goldman, Activation of the AMP-activated protein kinase by the anti-diabetic drug metformin in vivo. Role of mitochondrial reactive nitrogen species, J. Biol. Chem. 279 (2004) 43940–43951.
[31] B.J. Davis, Z. Xie, B. Viollet, M.H. Zou, Activation of the AMP-activated kinase by antidiabetes drug metformin stimulates nitric oxide synthesis in vivo by promoting the association of heat shock protein 90 and endothelial nitric oxide synthase, Diabetes 55 (2006) 496–505.