AMP-activated Protein Kinase Phosphorylates and Desensitizes Smooth Muscle Myosin Light Chain Kinase**

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Smooth muscle contraction is initiated by a rise in intracellular calcium, leading to activation of smooth muscle myosin light chain kinase (MLCK) via calcium/calmodulin (CaM). Activated MLCK then phosphorylates the regulatory myosin light chains, triggering cross-bridge cycling and contraction. Here, we show that MLCK is a substrate of AMP-activated protein kinase (AMPK). The phosphorylation site in chicken MLCK was identified by mass spectrometry to be located in the CaM-binding domain at Ser815. Phosphorylation by AMPK desensitized MLCK by increasing the concentration of CaM required for half-maximal activation. In primary cultures of rat aortic smooth muscle cells, vasoconstrictors activated AMPK in a calcium-dependent manner via CaM-dependent protein kinase kinase-β, a known upstream kinase of AMPK. Indeed, vasoconstrictor-induced AMPK activation was abrogated by the STO-609 CaM-dependent protein kinase kinase-β inhibitor. Myosin light chain phosphorylation was increased under these conditions, suggesting that contraction would be potentiated by abolition of AMPK. Indeed, in aortic rings from mice in which MLCK has been deleted, KCl- or phenylephrine-induced contraction was increased. The findings suggest that AMPK attenuates contraction by phosphorylating and inactivating MLCK. This might contribute to reduced ATP turnover in the tonic phase of smooth muscle contraction.

Vascular smooth muscle contraction can be initiated by agonists acting via G protein-coupled receptors (1), resulting in phospholipase Cβ activation and the formation of inositol 1,4,5-trisphosphate and diacylglycerol. Inositol 1,4,5-trisphosphate induces Ca2+ release from sarcoplasmic reticulum stores (2), whereas diacylglycerol activates protein kinase C (3). The rise in [Ca2+]i is further increased by Ca2+ influx through voltage-gated Ca2+ channels. Binding of Ca2+ to calmodulin (CaM) (4) activates smooth muscle myosin light chain kinase (smMLCK) by inducing a conformational change that removes an autoinhibitory domain from the kinase active site and allows smMLCK to phosphorylate 20-kDa regulatory myosin light chains (MLC) at Ser19 (5). MLC phosphorylation is necessary and sufficient for the initiation of contraction, triggering cross-bridge formation and contraction (6).

The activity of smMLCK itself can be modulated by phosphorylation, which changes its Vmax or affinity for Ca2+/CaM. smMLCK is phosphorylated at two sites (7, 8) by cAMP-dependent protein kinase (PKA). One site (site A, corresponding to Ser19 in chicken and Ser992 in rabbit smMLCK and conserved in the human, mouse, and rat sequences) lies in the C-terminal CaM-binding domain. Treatment with PKA inactivates MLCK by decreasing its affinity for Ca2+/CaM (9). Similar changes in affinity have also been observed after phosphorylation by Ca2+/CaM-dependent kinase II (7) and protein kinase C (10). Moreover, smMLCK contains several consensus sequences for proline-directed kinases, and phosphorylation by MAPK increases the Vmax with no change in affinity for Ca2+/CaM (10, 11).

The abbreviations used are: CaM, calmodulin; smMLCK, smooth muscle myosin light chain kinase; MLC, myosin light chain(s); PKA, cAMP-dependent protein kinase; MAPK, mitogen-activated protein kinase; AMPK, AMP-activated protein kinase; CaMKβ, Ca2+/calmodulin-dependent protein kinase β; ACC, acetyl-CoA carboxylase; MOPS, 4-morpholinepropane sulfonic acid; HPLC, high pressure liquid chromatography; ASM, aortic smooth muscle cell; ERK, extracellular signal-regulated kinase.
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AMPK-activated protein kinase (AMPK) acts as an energy sensor at both the cellular and systemic levels in mammals (12, 13). AMPK is a heterotrimer consisting of a catalytic α-subunit and two regulatory subunits, β and γ. Each subunit has multiple isoforms (α1, α2, β1, β2, γ1, γ2, and γ3), giving 12 possible combinations of holoenzyme with different tissue distribution and subcellular localization. AMPK can be activated by changes in the intracellular AMP/ATP ratio, which occurs, for example, under anoxia or other stresses (12), or via a rise in [Ca2+]. (13, 14). Activation by the upstream kinases LKB1 (Peutz-Jeghers protein) and CaMKKβ/CaM-dependent protein kinase kinase-β (CaMKKβ) occurs via phosphorylation of Thr172 in the activation loop of the catalytic α-subunits (13, 14). AMP not only allosterically stimulates AMPK, but also protects Thr172 against dephosphorylation by protein phosphatases (15). Therefore, changes in both intracellular Ca2+ and AMP concentrations could play separate or interdependent roles in the regulation of AMPK activity. Once activated, AMPK decreases ATP consumption and stimulates ATP-producing processes (12).

In this work, we show that smMLCK is a substrate of AMPK. Phosphorylation by AMPK decreased the affinity of smMLCK for Ca2+/CaM. We also show that stimulation of aortic smooth muscle cells by vasoconstrictors led to Ca2+-dependent AMPK activation, which was blocked by the CaMKKβ inhibitor. Moreover, in aortic rings of α1-AMPK knock-out mice, KCl- and phenylephrine-induced contraction was potentiated. The findings support the emerging notion that the role of AMPK extends beyond that of energy sensing.

EXPERIMENTAL PROCEDURES

Materials—Heterotrimeric recombinant bacterially expressed α1β1γ1-AMPK (16), bovine heart PKA catalytic subunits (17), chicken gizzard MLCK (18), and myosin II light chains (19) were purified as described previously. Sequencing grade trypsin (Promega); cell culture medium (Invitrogen); calmodulin, oligomycin, phenylephrine, CaM-agarose, anti-full-length MLCK antibody (clone K36), and anti-rabbit and anti-mouse IgG conjugated to peroxidase (Sigma); vasopressin, angiotensin II, anti-phospho-Ser221 ACC2 antibody (Bioke); anti-phospho-Ser211 ACC2 antibody (Upstate); enhanced chemiluminescence reagents (Thermo Fisher Scientific); anti-rabbit IgG conjugated to IRDye 800 (Rockland Immunocchemicals, Inc., Philadelphia); and anti-sheep IgG conjugated to Alexa 680 (Molecular Probes) were from the indicated sources. Bacterially expressed recombinant CaMKKβ was kindly donated by Professor D. Carling (Imperial College London). Anti-α1/α2-AMPK and anti-CaMKKβ antibodies were kindly provided by Professor D. G. Hardie (University of Dundee, Dundee, Scotland). Other reagents were from sources described previously (20, 21).

In Vitro Phosphorylation and Enzyme Assays—Recombinant bacterially expressed α1β1γ1-AMPK (20 μg) was activated by incubation in phosphorylation buffer (10 mM MOPS (pH 7), 10 mM magnesium acetate, 0.5 mM EDTA, and 0.1% (v/v) 2-mercaptoethanol) with 0.5 μg of recombinant bacterially expressed CaMKKβ, 0.1 mM MgATP, 10 μg/ml CaM, 1.5 mM CaCl2, 1 mM EGTA, 20 mM dithiothreitol, and 0.2 mM AMP in a final volume of 200 μl for 60 min at 30 °C. Chicken gizzard smMLCK (10 μg) was incubated in phosphorylation buffer with activated AMPK (15 milliunits) or PKA (5 milliunits) and 0.1 mM [γ-32P]MgATP (specific radioactivity, 500 cpm/pmol) in a final volume of 50 μl at 30 °C in the presence and absence of 10 μg/ml CaM. At the indicated times, aliquots (10 μl) were removed for SDS-PAGE and phosphorimaging for the measurement of 32P incorporation.

To study the effects of phosphorylation by AMPK and PKA on MLCK activity, chicken gizzard smMLCK (0.4 μg) was incubated in phosphorylation buffer with 0.1 mM MgATP in a final volume of 40 μl with no further additions or with AMPK (60 milliunits) or PKA (50 milliunits). After 10 min at 30 °C, the reaction mixtures were placed on ice and diluted 5-fold in phosphorylation buffer. Aliquots (2.5 μl) were assayed for MLCK in a final volume of 20 μl of phosphorylation buffer containing 10 μg/ml CaM, 0.1 mg/ml purified chicken gizzard MLC, 6 mM CaCl2, 5 mM EGTA, and 0.1 mM [γ-32P]MgATP (specific radioactivity, 500 cpm/pmol). After 4 min at 30 °C, aliquots (10 μl) were spotted on 1 × 1-cm squares of Whatman P-81 phosphocellulose paper and immersed in 75 mM phosphoric acid. After washing four times with phosphoric acid and once with acetone, the papers were dried and counted by Čerenkov radiation.

AMPK was assayed in polyethylene glycol fractions (22, 23) or in anti-α1/α2-AMPK antibody immunoprecipitates of cell lysates (16, 20). Purified recombinant activated AMPK (22) and purified PKA catalytic subunits (23) were assayed as indicated. One unit of protein kinase activity corresponds to the amount of enzyme that catalyzes the incorporation of 1 nmol of 32P/min into its substrate under the assay conditions.

Phosphorylation Site Identification by Mass Spectrometry—The phosphorylated band corresponding to MLCK phosphorylated in vitro by AMPK (as described above) was cut from Coomassie Blue-stained gels and digested with 1 μg of sequencing grade trypsin as described (24). Peptides were separated by reverse-phase narrow-bore HPLC at a flow rate of 200 μl/min, and radioactivity peaks were analyzed by nanoelectrospray ionization tandem mass spectrometry in an LCQ Deca XP Plus ion-trap mass spectrometer (Thermo Finnigan, San Jose, CA).

Pulldown Experiments with CaM-Agarose—smMLCK from chicken gizzard (0.5 μg) was incubated in phosphorylation buffer with 0.1 mM MgATP in a final volume of 50 μl with no other additions or with AMPK (60 milliunits) for 60 min at 30 °C. The reaction was incubated with CaM-agarose (20 μl of a 1:1 suspension) in the same buffer supplemented with 5 mM CaCl2 for 60 min at 30 °C. Following centrifugation, the pellet was boiled in SDS-PAGE sample buffer and analyzed by immunoblotting with anti-full-length MLCK as primary antibody. The same procedure was followed for centrifuged aortic smooth muscle cell (ASMC) extracts (150 μl).

Isolation of Aortic Explants and Treatment and Lysis of ASMCs—Primary rat ASMC cultures were prepared from aortic explants. Thoracic aortas from male Wistar rats were rapidly removed, cleaned of any adherent connective tissue, opened longitudinally, and rubbed along the inner surface to remove the endothelium. Aortas were then cut into squares (~10 mm2) and placed in 6-well microtiter plates in Dulbecco’s modified Eagle’s medium supplemented with 10% (w/v) fetal bovine
serum, 4 mM l-glutamine, 100 units/ml penicillin, and 100 μg/ml streptomycin. After 7–10 days, when suitable amounts of smooth muscle cells growing out of the explant were visible, explants were removed, and the cells were harvested in a tissue culture flask (175-cm² growth area). When they had reached confluence, the cells were passaged once before the experiment. The medium of early-passage ASMCs was replaced 24 h prior to treatment with Dulbecco’s modified Eagle’s medium without fetal bovine serum and containing a lower glucose concentration (1 g/liter). Cells were incubated with angiotensin II, vasopressin, or endothelin-1 at 10⁻⁷ M with or without a 60-min pretreatment with the CaMKKβ inhibitor STO-609 (10 μM) for the times indicated in the figures and legends. Control cells received an appropriate volume of relevant vehicle. For AMPK assays on immunoprecipitates and immunoblotting, cells were lysed in buffer containing 50 mM HEPES (pH 7.6), 50 mM KCl, 50 mM NaF, 5 mM sodium pyrophosphate, 1 mM EDTA, 1 mM EGTA, 1 mM dithiothreitol, 0.1% (w/v) Triton X-100, 5 mM β-glycerophosphate, and a mixture of protease inhibitors (Roche Applied Science). Lysates were centrifuged at 13,000 g for 10 min, and protein concentrations in the resulting supernatants were determined. For immunoblottling MLCC, cells were extracted in 8 M urea, 2% (w/v) Nonidet P-40, 15 mM β-mercaptoethanol, and a mixture of protease inhibitors.

**Immunoblotting**—Extracts were prepared as described above. Proteins were resolved by SDS-PAGE and electroblotted onto polyvinylidene difluoride membranes (Millipore). The membranes were probed with the following antibodies: anti-phospho-Thr172 AMPK, anti-phospho-Ser211 ACC2, anti-phospho-Thr202/Tyr204 ERK1/2, anti-phospho-Thr180/Tyr182 p38 MAPK, anti-phospho-Ser19 MLCK, and anti-full-length smMLCK protein, respectively, after 20 min of incubation. In both cases, phosphorylation was reduced by Ca²⁺/CaM (Fig. 1A), suggesting that the phosphorylation site(s) lie in the C-terminal CaM-binding domain. Indeed, phosphorylation by both PKA and AMPK increased the K₅₅ for CaM (Fig. 1B) from 0.04 μg/ml in the control to 0.17 in the PKA- and AMPK-treated samples (p < 0.05, n = 4).

After maximal phosphorylation by AMPK/[γ-³²P]Mg-ATP and SDS-PAGE, the smMLCK band was digested with trypsin; peptides were separated by reverse-phase HPLC; and the major radioactive peak (Fig. 2A) was analyzed by mass spectrometry. Ser²⁰⁵ was identified as the phosphorylated residue in the phosphorylated peptide (⁶⁴LSSMAMISGMSGR⁷⁵). Ser²⁰⁵ corresponds to site A in the C-terminal CaM-binding domain of smMLCK (Fig. 2B), known to be phosphorylated by PKA (9), Ca²⁺/CaM-dependent kinase II (7), and protein kinase C (28). The lower stoichiometry of phosphorylation of smMLCK by PKA and AMPK compared with PKA (Fig. 1A), suggesting that the phosphorylation site(s) lie in the C-terminal CaM-binding domain. Indeed, phosphorylation by both PKA and AMPK increased the K₅₅ for CaM (Fig. 1B) from 0.04 μg/ml in the control to 0.17 in the PKA- and AMPK-treated samples (p < 0.05, n = 4).

**Other Methods**—Protein concentration was estimated by the method of Bradford (27) with bovine serum albumin as a standard. **Statistical Analyses**—Data are expressed as the means ± S.E. Student’s two-sided t test or one-way analysis of variance (Bonferroni adjustment) was used to assess the statistical significance (p < 0.05) of the data.

**RESULTS**

**In Vitro Experiments**—smMLCK was identified serendipitously as a potential AMPK target by mass spectrometric analysis of liver proteins eluting from a CaM-Sepharose column and containing a M₉, 120,000 gel band phosphorylated in vitro by AMPK. We therefore investigated whether smMLCK might be a bona fide AMPK substrate and whether phosphorylation affected its activity. Upon incubation with [γ-³²P]Mg-ATP and activated AMPK or PKA catalytic subunits, purified chicken gizzard smMLCK was phosphorylated to a stoichiometry of 0.35 or 0.75 mol of phosphate incorporated per mol of smMLCK protein, respectively, after 20 min of incubation. In both cases, phosphorylation was reduced by Ca²⁺/CaM (Fig. 1A), suggesting that the phosphorylation site(s) lie in the C-terminal CaM-binding domain. Indeed, phosphorylation by both PKA and AMPK increased the K₅₅ for CaM (Fig. 1B) from 0.04 μg/ml in the control to 0.17 in the PKA- and AMPK-treated samples (p < 0.05, n = 4).

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**Experiments in ASMCs**—AMPK activation by vasoconstrictors was tested in primary cultures of ASMCs generated from rat aortic explants. AMPK was immunoprecipitated with anti-
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α1- and anti-α2-AMPK antibodies prior to AMPK assay. The AMPK catalytic α1-subunit isoform accounted for 90% of the total AMPK activity in ASMC lysates (data not shown), confirming results obtained with porcine carotid arteries (29). Treatment with \(10^{-7} \text{M} \) vasopressin, angiotensin II, or endothelin-1 led to AMPK activation (Fig. 3A). Phenylephrine treatment of ASMCs did not lead to AMPK activation, presumably because of the disappearance of α1-adrenergic receptors in these cultured cells. Because \(\text{Ca}^{2+} \) mediates vasoconstrictor action, AMPK activation was measured in ASMCs incubated with \(\text{Ca}^{2+} \text{-ionophore. When measured in polyethylene glycol fractions of cell lysates, AMPK was activated \sim 2 \text{-fold after 2 min of treatment with ionophore (Fig. 3B). It is noteworthy that at this concentration of A23187, adenine nucleotide concentrations were shown previously to be unmodified (30), suggesting that AMPK is probably not activated via a rise in [AMP] through the LKB1 pathway. Moreover, AMPK activation induced by vasopressin was blocked by 5 mM EGTA in the incubation medium (Fig. 3C).}

An obvious candidate for mediating vasoconstrictor-induced AMPK activation in ASMCs is CaMKK\(\beta \), an upstream kinase in the AMPK cascade (13, 14). The CaMKK\(\beta \) gene codes for several splice variants. By probing with a polyclonal antibody that recognizes CaMKK\(\beta \), ASMC extracts were shown to contain two immunoreactive species (Fig. 4): a major band (M, 65,000) corresponding to the CaMKK\(\beta 1 \) isoform predominantly expressed in normal rat brain and a minor band (M, 60,000) corresponding to the CaMKK\(\beta 3 \) isoform predominantly expressed in HeLa cells (31).

STO-609 is a relatively selective and cell-permeable inhibitor of CaMKK\(\beta \) (32). At a concentration of 10 µM, STO-609 completely blocked AMPK activation by vasopressin, angiotensin II, and endothelin-1 (Fig. 3A). Immunoblotting of ASMC lysates indicated that the effect of STO-609 on AMPK activity was due to a decrease in Thr\(^{172} \) AMPK α-subunit phosphorylation, with no change in total AMPK α-subunit expression (Fig. 3A).

Because the MAPK pathway is known to be activated in ASMCs by agonists acting via G protein-coupled receptors (33), we examined ERK1/2 and p38 MAPK activation in comparison with AMPK activation in response to vasopressin by immunoblotting with activation loop phospho-specific antibodies (supplemental Fig. 1). Phosphorylation of these protein kinases was transient, with maximal phosphorylation of ERK and p38 MAPK at 5 min preceding that of AMPK and its substrate ACC2 (supplemental Fig. 1). To test the potential involvement of the CaMKK\(\beta \)/AMPK and MAPK cascades in MLC phosphorylation, ASMCs were treated with vasopressin in the presence or absence of STO-609, PD98059, or SB203580, inhibitors of CaMKK\(\beta \), ERK1/2, and p38 MAPK, respectively. As expected, incubation with vasopressin led to an increase in MLC Ser\(^{19} \) phosphorylation (Fig. 5), which was significant after 30 min of treatment. Preincubation with STO-609 accelerated MLC phosphorylation, which became maximal after 5 min of treatment with vasoconstrictor (Fig. 5). By contrast, abrogation of ERK1/2 and p38 MAPK activation with PD98059 and SB203580, respectively, had no effect on the time course of MLC phosphorylation (data not shown), suggesting that antagonism of the CaMKK\(\beta \)/AMPK axis is solely responsible for the rise in MLC phosphorylation in response to vasopressin treatment. A decreased affinity of smMLCK for CaM induced by vasopressin treatment was demonstrated using CaM-agarose pulldown experiments and analysis of the pellet by immunoblotting with anti-full-length smMLCK as primary antibody (Fig. 6). The smMLCK signal decreased in extracts from vasopressin-treated ASMCs, but this effect was absent after prein-
cubation with STO-609 (Fig. 6), suggesting that the CaMKK/AMPK axis is mediating the decreased affinity of smMLCK for CaM induced by vasoressin.

Experiments in α1-AMPK Knock-out Mice—As the α1-subunit isoform accounted for 90% of the total AMPK in vascular smooth muscle, we used α1-AMPK knock-out mice (34). The major phenotypic change observed in α1-AMPK knock-out mice is splenomegaly (35), but there were no differences in the concentrations of adrenaline, noradrenaline, or dopamine in urine compared with control mice. The functional responses of aorta induced to contract by depolarization with KCl (10 rings/five mice) or by treatment with phenylephrine (14 rings/seven mice) were studied (Fig. 7). Because AMPK is known to phosphorylate endothelial nitric-oxide synthase, the rings were treated with the nitric-oxide synthase inhibitor L-NAME. The maximal extent of force developed in aortic rings in response to increasing concentrations of phenylephrine was significantly greater in the α1-AMPK knock-out mice than in the wild-type controls (Fig. 7A), the sensitivity to phenylephrine being the same, however, in both groups. The increased contraction observed in α1-AMPK knock-out mice was also seen in rings depolarized with increasing concentrations of KCl (Fig. 7B). In addition, the increase in 

was increased by phenylephrine treatment (Fig. 7C) or by KCl-induced depolarization (Fig. 7D) produced a greater increase in tension development in rings from knock-out compared with wild-type mice, and this increased contractility in the α1-AMPK knock-out mice persisted in the presence of the calcium ionophore ionomycin (data not shown). These findings further support the contention that AMPK activation in vascular smooth muscle imparts a brake on contraction.

The effect of KCl-induced depolarization on AMPK activity was assessed in ASMCs by monitoring ACC2 phosphorylation. An increase in the extent of phosphorylation of ACC2 was seen after 5 min of treatment with KCl solution (143 mM KCl, 1.2 mM CaCl2, 1.2 mM MgCl2, 11.6 mM HEPES, and 11.5 mM glucose) (supplemental Fig. 2). Moreover, ACC2 phosphorylation was prevented when the cells were preincubated with STO-609. However, phenylephrine was without effect in ASMCs, as neither AMPK activation nor MLCK phosphorylation was seen with this agonist (data not shown).

**DISCUSSION**

The conclusion that AMPK participates in the control of smooth muscle contraction is based on the following: (i) vasoconstrictors activated AMPK in ASMCs; (ii) AMPK phosphorylated smMLCK and reduced its affinity for CaM; (iii) inhibition of CaMKK antagonized vasoconstrictor-induced AMPK activation and smMLCK desensitization and resulted in enhanced MLCK phosphorylation; and (iv) aortic contractility due to KCl-induced depolarization or phenylephrine treatment was increased in α1-AMPK knock-out mice. Together, the data suggest that the CaMKK/AMPK axis modulates Ca2+-dependent contraction via AMPK-induced phosphorylation of smMLCK.

Ser815 was identified as the phosphorylation site for AMPK in smMLCK. The sequence surrounding Ser815 of chicken smMLCK is conserved (Fig. 2B) and perfectly fits the canonical

B. Viollet, unpublished data.

![Identification of the smMLCK phosphorylation site for AMPK.](image)
AMPK substrate recognition motif, \( \phi(X,\beta)(S/T)XXX\phi \), where \( \phi \) is a hydrophobic residue (Met, Val, Leu, Ile, or Phe), \( \beta \) is a basic residue (Arg, Lys, or His), and the parentheses indicate that the order of residues at the P-4 and P-3 positions is not critical (36).

It was recently reported that in *Drosophila melanogaster* in which its single AMPK catalytic subunit isoform had been deleted, epithelial cell polarity was disrupted in a manner similar to the knock-out of LKB1. Moreover, AMPK was proposed to directly phosphorylate MLC at the same residue phosphorylated by smMLCK, thereby participating in the establishment of cell polarity (37). However, in our hands, treatment of ASMCs with 5-amino-4-imidazolecarboxamide riboside or the A-769662 (Abbott) AMPK activator (4) led to AMPK activation, which was not associated with an increase in MLC phosphorylation (supplemental Fig. 3).

In blood vessels, metabolic stress or hypoxia can induce vasodilatation, increasing oxygen availability in peripheral tissues. Endothelium-derived relaxing factors probably play a significant role in this respect. AMPK, which is activated by hypoxia due to the rise in AMP, could mediate vasodilatation via phosphorylation-induced activation of endothelial nitric-oxide synthase, whereby increasing NO production (38). In blood vessels devoid of endothelium, AMPK activation due to metabolic...
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stress or anoxia was found to decrease force generation in response to endothelin-1 treatment (29). The underlying mechanisms, which were not investigated, are in line with our findings. Ca\(^{2+}\)/CaM-dependent kinase II has also been implicated in the desensitization of MLCK to Ca\(^{2+}\)/CaM stimulation, but the Ca\(^{2+}\)/CaM-dependent kinase II inhibitor KN-93 was shown not to impair phenylephrine-induced contraction (39).

The AMPK catalytic subunit isoform predominant in vascular smooth muscle is α1 (29), and our results obtained with α1-AMPK knock-out mice support the notion that AMPK activation would attenuate smooth muscle contraction. In addition, it was recently shown that smooth muscle α1-AMPK subunit activation induces vasorelaxation of mouse aorta independent of NO and the presence of endothelium (40), again in line with our findings. Unlike cardiac or skeletal muscles, vascular smooth muscle can maintain tonic force with low ATP turnover in the continued presence of vasoconstrictor. Although force initiation is associated with a rise in [Ca\(^{2+}\)], and smMLCK activation, during the tonic phase of contraction, [Ca\(^{2+}\)] \(</\) falls even though tension is maintained (41). Despite the decline in [Ca\(^{2+}\)], and smMLCK activity, MLC phosphorylation and muscle contraction are maintained, suggesting that Ca\(^{2+}\)-independent contractile events participate in agonist-induced sustained contraction and MLC phosphorylation. Here, we provide evidence that AMPK is a modulator of vasoconstrictor-induced smooth muscle contraction in the absence of metabolic stress. In conclusion, our results suggest that AMPK attenuates contraction by phosphorylating and inactivating smMLCK and that this might contribute to reduced ATP turnover in the tonic phase of smooth muscle contraction.

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