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The role of the calponin homology domain of smoothelin-like 1 (SMTNL1) in myosin phosphatase inhibition and smooth muscle contraction

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Abstract

In this study, we provide further insight into the contribution of the smoothelin-like 1 (SMTNL1) calponin homology (CH)-domain on myosin light chain phosphatase (SMPP-1M) activity and smooth muscle contraction. SMTNL1 protein was shown to have inhibitory effects on SMPP-1M activity but not on myosin light chain kinase (MLCK) activity. Treatment of β-escin permeabilized rabbit, ileal smooth muscle with SMTNL1 had no effect on the time required to reach half-maximal force ($t_{1/2}$) during stimulation with pCa6.3 solution. The addition of recombinant SMTNL1 protein to permeabilized, smooth muscle strips caused a significant decrease in contractile force. While the calponin homology (CH)-domain was essential for maximal SMTNL1-associated relaxation, it alone did not cause significant changes in force. SMTNL1 was poorly dephosphorylated by PP-1C in the presence of the myosin targeting subunit (MYPT1), suggesting that phosphorylated SMTNL1 does not possess “substrate trapping” properties. Moreover, while full-length SMTNL1 could suppress SMPP-1M activity toward LC_{20} in vitro, truncated SMTNL1 lacking the CH-domain was ineffective. In summary, our findings suggest an important role for the CH-domain in mediating the effects of SMTNL1 on smooth muscle contraction.

Keywords

CH-domain; Smoothelin-like 1; CHASM; Smooth muscle; Calcium desensitization; Myosin light chain phosphatase; MYPT1

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Introduction

Smoothelin-like 1 protein (SMTNL1), originally called CHASM (*c*alponin *h*omology-*a*ssociated *s*mooth *m*uscle) [1], shares significant sequence similarity with the smoothelin family of smooth muscle proteins. SMTNL1 was identified as a novel ~60 kDa protein phosphorylated during 8-bromo-cGMP-induced relaxation of ileal smooth muscle [1]. It is now recognized that SMTNL1 expression is not limited strictly to smooth muscle; the protein is also expressed in type 2a striated muscle fibers [2]. SMTNL1 contributes to cAMP-/cGMP-mediated changes in contractility in both skeletal and smooth muscle. Cyclic nucleotides can relax smooth muscle without a comparable change in intracellular calcium levels ($[Ca^{2+}]_i$) [3,4]. This phenomenon, whereby a decrease in myosin regulatory light chain (LC_{20}) phosphorylation and subsequent force occurs without a proportionate decline in $[Ca^{2+}]_i$, is referred to as 'Ca^{2+} desensitization'. Our previous results demonstrated that cGMP and/or cAMP-activated kinases could mediate SMTNL1 phosphorylation in vitro, and mutation of Ser-301 to Ala completely abolished this phosphorylation [1]. Furthermore, SMTNL1 was phosphorylated in vivo on Ser-301 in response to adrenergic signals in both skeletal and smooth muscle [2]. Aortas from *smtnl*$^{-/-}$ mice exhibit enhanced vasorelaxation before exercise and greatly attenuated contractile responses to α-adrenergic agonists. *Smtn*l$^{-/-}$ mice also exhibited increased accumulation of type 2a skeletal muscle fibers before exercise training and better performance after forced endurance training [2]. Thus, the SMTNL1 protein is currently proposed to act as a physiological regulator of contractile activity, the activity of which can be modulated through cGMP/cAMP-mediated phosphorylation of Ser-301.

SMTNL1 contains a highly conserved calponin homology (CH)-domain in the C-terminal portion of the protein [5]. CH-domains have been identified in a variety of cytoskeletal and signaling proteins that are known to play key regulatory roles in muscle contraction [6–8]. The SMTNL1 CH-domain could not mediate an interaction of the protein with F-actin in vitro [1]; however, the domain was recently hypothesized to be an important modulator of SMTNL1 effects on myosin dephosphorylation by myosin phosphatase (SMPP-1M) [2]. In addition, the NMR solution structure of the SMTNL1 CH-domain was recently solved [5]. The SMTNL1 CH-domain can be distinguished from other CH-domains by a unique C-terminal tail that contains a calmodulin (CaM)-binding IQ-motif. In the present study, we provide further insight into the contribution of the SMTNL1 CH-domain to the effects of the protein on smooth muscle contraction.

Experimental procedures

Plasmid generation and bacterial expression of smoothelin-like 1 constructs

The full-length human smoothelin-like 1 (SMTNL1) cDNA was generated from I.M.A.G.E. clone 3593616 as previously described [1]. Constructs encoding SMTNL1 variants were produced by amplification of fragments with standard PCR techniques and primers designed to introduce proper restriction sites for subcloning. Fragments of SMTNL1 encoding either the carboxyl-terminal CH-domain of SMTNL1 (SMTNL1-CH; aa 346–459) or a truncated SMTNL1 protein that lacked the CH-domain (SMTNL1-ΔCH; aa 1–346) were subcloned into BamHI/NotI sites of the pGEX-6P1 vector (GE Healthcare). All constructs were verified by DNA sequencing. Recombinant proteins were produced in *E. coli* (DE3). GST-fusion proteins were isolated using glutathione-Sepharose 4B. In some cases, GST-fusion proteins were cleaved 'on-column' by treatment with PreScission Protease as described by the manufacturer (GE Healthcare). Proteins used for experiments with isolated smooth muscle were exchanged into 30 mM PIPES, pH 7.1, 165 mM potassium methane sulfonate, and 5 mM magnesium sulfonate and concentrated with an Amicon centrifugal filter (Millipore).

Muscle tension experiments in isolated rabbit ileum

Muscle strips (3 mm × 250 μM) of rabbit longitudinal ileal smooth muscle were attached to a force transducer (SensorOne AE801, Sausalito, CA) with monofilament surgical silk and stretched to 1.3× resting length [9]. The strips were immersed in a small well on a stir plate and equilibrated for 30 min in normal extracellular solution (NES). After obtaining a good contractile response to high K^+ extracellular solution (154 mM K^+; KES), muscle strips were permeabilized by incubation with 50 μM *β*-escin for 30 min in an intracellular solution (G1) with 10 μM A23187 added for the final 10 min to deplete intracellular Ca^{2+} stores. The composition of G1 solution was (in mM): 10 creatine phosphate (Na_2CP), 5.16 adenosine triphosphate (Na_2ATP), 7.31 magnesium methanesulfonate ($MgMS_2$), 74.1 potassium methanesulfonate (KMS), 1 ethylenebis(oxyethylenenitrilo)tetraacetic acid (K_2EGTA). Maximal contraction was obtained with maximal calcium (pCa 4.5) before and after the protocol in all experiments. Solutions with desired free Ca^{2+} levels (expressed as pCa) were obtained by mixing G10 and CaG solutions. The composition of CaG solution was (in mM): 10 Na_2CP, 5.14 Na_2ATP, 7.25 $MgMS_2$, 47.1 KMS, 10 $K_2CaEGTA$, and the composition of G10 solution was (in mM): 10 Na_2CP, 5.14 Na_2ATP, 7.92 $MgMS_2$, 46.6 KMS, 10 K_2EGTA. The force levels obtained with relaxing solution (G1) and pCa 4.5 were designated as 0% and 100%, respectively.

To determine whether the SMTNL1-induced desensitization to Ca^{2+} was mediated by inhibition of MLCK or stimulation of SMPP-1M activity, we measured the effect of SMTNL1 on the rate of contraction in *β*-escin permeabilized rabbit ileum smooth muscle in which SMPP-1M activity was blocked. ATP*γ*S was used as a substrate, because LC_{20} thiophosphorylation resists dephosphorylation [10,11]. Ileal smooth muscle strips were incubated at 15°C in Ca^{2+}-free, ATP-free solution (G10′) for 20 min to deplete endogenous ATP. The strips were then transferred to ATP-free pCa6.2 (pCa6.2′) solution with or without SMTNL1 (10 μM) for 5 min. Subsequently, contraction was initiated by addition of ATP*γ*S (2 mM). The rate of contraction was estimated by analyzing the time required for the muscle tension to increase by 50% of maximum ($t_{1/2}$). The force levels obtained upon addition of ATP*γ*S and at the plateau of contraction were designated as 0 and 100%, respectively.

LC_{20} kinase and phosphatase assays

Purified, smooth muscle MLCK (2.5 ng) was assayed against chicken gizzard LC_{20} (0.1 mg) in a buffer containing 25 mM HEPES, pH 7.5, 25 mM $MgCl_2$, 2 mM $CaCl_2$, 5 μM calmodulin, 10 μM microcystin, 1 mM DTT, 0.5 mM [γ^{-32}P]-ATP (20,000 cpm/nmol) with increasing concentrations of SMTNL1. LC_{20} phosphorylation was measured by terminating the kinase reactions with ice-cold 25 % (w/v) trichloroacetic acid; BSA was added as a carrier protein, and the reactions were centrifuged to precipitate the proteins. After the pellets were washed extensively, ^{32}P incorporation onto LC_{20} was measured by scintillation counting.

Two substrate proteins were used for SMPP-1M assays: chicken gizzard LC_{20} and recombinant SMTNL1. Radiolabeled, phospho-LC_{20} (2 mg) was generated in a buffer containing 25 mM HEPES, pH 7.5, 2.5 mM $MgCl_2$, 2 mM $CaCl_2$, 5 μM calmodulin, 0.1 mg MLCK, 10 μM microcystin, 1 mM DTT, and 0.5 mM [γ^{-32}P]ATP (0.2 mCi). Recombinant GST-SMTNL1 (1 mg) was phosphorylated with PKA catalytic subunit (3 μg) in a buffer containing 25 mM HEPES, pH 7.5, 2.5 mM $MgCl_2$, 10 μM microcystin, 1 mM DTT and 0.5 mM [γ^{-32}P]ATP (0.2 mCi). Unincorporated [γ^{-32}P]ATP was removed by extensive dialysis against 25 mM Tris, pH 7.5, 1 mM EDTA, and 1 mM DTT. Phosphatase assays were carried out as previously described [12]. Assay components included 0.4 nmol purified PP-1C (rabbit skeletal muscle) in the presence or absence of 1 nmol recombinant MYPT1 (chicken; M130 isoform).

Statistics

All values are means ± standard error of the mean (S.E.M.) unless otherwise indicated. The Student's *t*-test was used to determine statistical significance with $P < 0.05$ considered to be significant.

Results

Relaxation of Ca^{2+}-induced tension by recombinant SMTNL1 proteins

We have previously shown that full-length, recombinant SMTNL1 protein can induce a concentration-dependent relaxation of contractile tension under submaximal calcium levels (i.e., pCa 6.3) [1]. Recombinant proteins were generated (Fig. 1) to test the functional relevance of the CH-domain on the effects of SMTNL1 on muscle contractility. As shown in Fig. 2, the addition of SMTNL1-ΔCH protein (10 μM) that lacks the carboxy-terminal CH-domain induced a relaxation of 7.7 ± 3.5% *(n* = 4), a value nearly 70% less than the relaxation evoked by the full-length SMTNL1-WT protein, 25.4 ± 3.0% (*n* = 9). To determine whether the CH-domain alone was sufficient to evoke Ca^{2+}-desensitization of contractile force, a recombinant protein consisting only of the calponin homology domain from SMTNL1 was generated. The 13 kDa, SMTNL1-CH protein elicited a 10.0 ± 1.5% *(n* = 10) reduction in contractile force generated by pCa 6.3 solution.

SMTNL1 effects on MLCK and SMPP-1M activities

Ca^{2+} desensitization elicited by SMTNL1 in permeabilized smooth muscle strips could result from MLCK inhibition or SMPP-1M activation. We determined the rate of contraction upon administration of ATPγS since LC_{20} thiophosphorylation resists dephosphorylation by SMPP-1M [13]. Treatment with SMTNL1 had no effect on the time course of smooth muscle contraction. There was no difference in the time required to reach half-maximal force ($t_{1/2}$) when β-escin permeabilized ileal strips were incubated in the presence or absence of SMTNL1-WT protein, 1.5 ± 0.15 min versus 1.4 ± 0.04 min, respectively (Fig. 3a). These data suggest that SMTNL1-induced Ca^{2+} desensitization was not mediated through an inhibition of MLCK activity. Moreover, when purified MLCK was assayed in vitro with increasing amounts of SMTNL1 (0–20 μM), no effect on the rate of LC_{20} phosphorylation was observed (Fig. 3b).

The effect of SMTNL1 on SMPP-1M activity was also investigated under in vitro conditions. PP-1C and SMPP-1M activity were measured using phosphorylated LC_{20} as a substrate. The addition of GST-SMTNL1 had a small positive effect on the activity of PP-1C toward phosphorylated LC_{20} (Fig. 4a). The effect of GST-SMTNL1 on SMPP-1M (PP-1C and MYPT1 combined in 0.4:1 molar mixture) was also measured using phosphorylated LC_{20} as a substrate. GST-SMTNL1 inhibited SMPP-1M activity in a concentration-dependent manner (Fig. 4b). Approximately, 55% of the SMPP-1M activity was inhibited with addition of 2.5 μM GST-SMTNL1. Further increases in GST-SMTNL1 (up to 20 μM) resulted in 75% inhibition of SMPP-1M activity. The addition of filtrate solution had no effect on PP-1C or SMPP-1M activity.

To eliminate the possibility that the effects of GST-SMTNL1 were due to the GST moiety, SMPP-1M activity was also assayed in the presence of Precission Protease-cleaved SMTNL1 proteins to further verify that the effects of GST-SMTNL1 were SMTNL1-induced. Indeed, we observed that the GST moiety had a positive influence on SMPP-1M activity toward phosphorylated LC20 (Fig. 4c). The effect of SMTNL1-WT, SMTNL1-ΔCH or SMTNL1-CH on SMPP-1M activity was measured. While SMTNL1-WT still retained its inhibitory potential toward SMPP-1M, neither SMTNL1-ΔCH nor SMTNL1-C proteins were able to inhibit SMPP-1M in vitro. The inhibitory effects of various SMTNL1 proteins on SMPP-1M activity are summarized in Fig. 4c.

The possibility that phosphorylated SMTNL1 might be a substrate for SMPP-1M and/or PP-1C was investigated. The activities of PP-1C and SMPP-1M (PP-1C with MYPT1 targeting subunit) were monitored in vitro using phosphorylated ^{32}P-SMTNL1-WT and phosphorylated ^{32}P-LC_20 for comparison. Consistent with previously published data [2], PP-1C in the absence of the MYPT1 targeting protein displayed low phosphatase activity toward phosphorylated LC_{20} (Fig. 5a). The addition of MYPT1 protein enhanced the ability of PP-1C to dephosphorylate LC_{20}. PP-1C could dephosphorylate ^{32}P-labeled SMTNL1-WT protein (Fig. 5b); however, the activity of PP-1C toward SMTNL1 was slightly less than that observed for ^{32}P-labeled LC_{20}. The addition of MYPT1 significantly reduced the PP-1C phosphatase activity toward ^{32}P-labeled SMTNL1-WT.

Discussion

Ca^{2+} desensitization and smooth muscle relaxation result from inhibition of myosin light chain kinase (MLCK) or activation of myosin light chain phosphatase (SMPP-1M) [3]. Cyclic nucleotide-induced phosphorylation and the inhibition of MLCK were originally proposed to promote Ca^{2+} desensitization. MLCK phosphorylation was associated with a decrease in calmodulin-binding affinity that in turn leads to a reduction in MLCK activity. However, observations that 8-Br-cGMP could accelerate dephosphorylation of LC_{20} when MLCK activity was inhibited demonstrated that enhancement of SMPP-1M activity was responsible for cyclic nucleotide-induced Ca^{2+} desensitization [10,13]. Indirect mechanisms that involve accessory proteins can contribute to the regulation of SMPP-1M activity and smooth muscle relaxation. For example, roles for HSP20 [14], telokin [15] as well as SMTNL1 [1,2] in the regulation of LC_{20} dephosphorylation by SMPP-1M and Ca^{2+} desensitization have been identified.

In the present study, SMTNL1 did not have any effect on MLCK-dependent phosphorylation of LC_{20} in vitro, nor did SMTNL1 cause a reduction in the rate of ATPγS-induced smooth muscle contraction. The latter event would be expected if MLCK-mediated LC_{20} phosphorylation was inhibited by SMTNL1 in smooth muscle. Our in vitro experiments may lack important regulatory components that are present within the permeabilized smooth muscle system. In situ experiments within permeabilized smooth muscle strips might also be influenced by the loss of SMTNL1-binding proteins or other regulatory proteins. Regardless of these issues, our results currently implicate a mechanism of contractile regulation that excludes SMTNL1 effects on MLCK. It appears that the primary effect of SMTNL1 on muscle contraction is through the regulation of SMPP-1M activity and myosin dephosphorylation.

It may be difficult to reconcile our findings that demonstrate SMTNL1-dependent relaxation of smooth muscle in situ and inhibition of SMPP-1M activity in vitro. One would expect that SMTNL1 would activate isolated SMPP-1M activity in biochemical experiments since the protein is able to relax isolated, permeabilized muscle strips. However, it is possible that cGMP-mediated phosphorylation of SMTNL1 alters its localization in the cell. Ultimately, if phosphorylated SMTNL1 was no longer present at the contractile apparatus, its inhibitory effects on SMPP-1M activity would be relieved and muscle relaxation could occur. The influence of phosphorylation on the cellular localization of SMTNL1, as well as its interaction with other proteins, will be required to address this possibility. Some cGMP-dependent phosphorylation events that relieve inhibitory potential have been previously identified in the regulation of smooth muscle contraction. For example, cGMP-dependent protein kinase (PKG) can directly phosphorylate the myosin phosphatase targeting subunit (MYPT1) of SMPP-1M, not to alter its activity toward phosphorylated LC_{20} but to prevent subsequent inhibition of SMPP-1M by zipper-interacting protein kinase and Rho-associated protein kinase [16,17].

A key regulatory determinant of SMTNL1 action appears to be its phosphorylation at Ser-301 [1,2]. It has been shown that the phosphorylation of SMTNL1 can inhibit SMPP-1M activity toward whole smooth muscle myosin [2]. This study by Wooldridge et al. also reported that SMTNL1 had no effect on purified, pig bladder SMPP-1M activity toward LC_{20} under in vitro conditions. Data presented in the present study suggest that there is some in vitro activity toward LC_{20} by an SMPP-1M complex containing PP-1C and chicken gizzard M130 MYPT1 isoform. The differences in the results may depend on the nature of the SMPP-1M holoenzyme used in the two studies. Additional experiments in this study demonstrated that radiolabeled ^{32}P-SMTNL1 was not a viable in vitro substrate for SMPP-1M; therefore, it would appear that SMTNL1 effects on myosin dephosphorylation do not occur through a direct inhibition of SMPP-1M activity. SMTNL1 apparently alters the diphosphorylation potential of myosin through interactions with the substrate protein itself and not SMPP-1M.

SMTNL1effectsonmuscle contractility may be mediated through its interactions with the contractile filament. SMTNL1 contains a single type-2 CH-domain at the C-terminus that shares sequence identity with the smoothelin family of smooth muscle specific proteins. CH-domains are present in a number of actin-binding proteins [8], but it is still unclear if the CH-domain is integral for the association of smoothelin proteins with actin filaments [18,19]. While we have already concluded that the CH-domain of SMTNL1 does not allow for associations with F-actin in vitro [1], the domain could act as a platform for interactions with other contractile proteins. For example, our recent structural analysis of the CH-domain of SMTNL1 uncovered the presence of an IQ-motif that imparts a unique apo-CaM-binding mode [5]. However, the equilibrium Ca^{2+}-binding constants for apo-CaM were unaltered in the presence of the SMTNL1 CH-domain (J. Gifford, H. Vogel and J. MacDonald unpublished data). While SMTNL1 has been hypothesized to act as a targeting protein in localizing apo-CaM to a discrete cellular compartment, it appears that the protein may not serve to dampen MLCK activity and contractile force by altering the muscle response to local changes in [Ca^{2+}]. The precise physiological role of the SMTNL1-CaM interaction and the effect of Ser-301 phosphorylation remain to be defined. Certainly, CaM-binding might influence the phosphorylation efficiency of SMTNL1, or alternatively, SMTNL1 phosphorylation may influence the CaM binding efficiency or Ca^{2+} association and/or dissociation of CaM. Moreover, it is possible that the CH-domain is required for the formation of SMTNL1 complexes with other proteins.

Based on the biochemical analyses and contractile responses obtained from our investigation of SMTNL1 variants, we can conclude that the CH-domain of SMTNL1 is required for maximal action in smooth muscle. Addition of recombinant SMTNL1-ΔCH was not sufficient for maximal Ca^{2+} desensitization, rather the full-length SMTNL1 protein was required. The importance of the CH-domain was also demonstrated in the ability of the various SMTNL1 proteins to inhibit SMPP-1M activity; only full-length SMTNL1 could retard phosphatase activity. Future studies might focus on selective mutation of residues within the CH-domain (e.g., the IQ-motif) to determine if this portion of SMTNL1 has specific effects on contractile activity. It has been reported that CH-domains can interface with a variety of proteins involved in cytoskeletal dynamics and/or signal transduction [8], and the CH-domain of SMTNL1 may enable an interaction with SMPP-1 M or other contractile proteins to permit the regulation of LC_{20} dephosphorylation in smooth muscle. Future experiments will also necessitate the use of intact models of muscle to examine the functional role of various SMTNL1 domains. Such experiments could rely on the introduction of plasmids encoding SMTNL1 variants by reversible permeabilization followed by tissue incubation to allow for expression of the proteins [20].

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References

1. Borman MA, MacDonald JA, Haystead TA. Modulation of smooth muscle contractility by CHASM, a novel member of the smoothelin family of proteins. FEBS Lett 2004;573:207–213.10.1016/j.febslet.2004.08.002 [PubMed: 15327999]
2. Wooldridge AA, Fortner CN, Lontay B, Akimoto T, Neppl RL, Facemire C, Datto MB, Kwon A, McCook E, Li P, Wang S, Thresher RJ, Miller SE, Perriard JC, Gavin TP, Hickner RC, Coffman TM, Somlyo AV, Yan Z, Haystead TAJ. Deletion of the protein kinase A/protein kinase G target SMTNL1 promotes an exercise-adapted phenotype in vascular smooth muscle. J Biol Chem 2008;283:11850–11859.10.1074/jbc.M708628200 [PubMed: 18310078]
3. Somlyo AP, Somlyo AV. Ca2+ sensitivity of smooth muscle and nonmuscle myosin II: modulated by G proteins, kinases, and myosin phosphatase. Physiol Rev 2003;83:1325–1358. [PubMed: 14506307]
4. Schlossmann J, Feil R, Hofmann F. Signaling through NO and cGMP-dependent protein kinases. Ann Med 2003;35:21–27.10.1080/07853890310004093 [PubMed: 12693609]
5. Ishida H, Borman MA, Ostrander J, Vogel HJ, MacDonald JA. Solution structure of the calponin homology (CH) domain from the smoothelin-like 1 protein: a unique apocalmodulin-binding mode and the possible role of the C-terminal type-2 CH-domain in smooth muscle relaxation. J Biol Chem 2008;283:20569–20578.10.1074/jbc.M800627200 [PubMed: 18477568]
6. Lorenzi M, Gimona M. Synthetic actin-binding domains reveal compositional constraints for function. Int J Biochem Cell Biol 2008;40:1806–1816.10.1016/j.biocel.2008.01.011 [PubMed: 18296101]
7. Friedberg F. Alternative splicing for members of human mosaic domain superfamilies. I. The CH and LIM domains containing group of proteins. Mol Biol Rep. 200810.1007/s11033-008-9281-9
8. Gimona M, Djinovic-Carugo K, Kranewitter WJ, Winder SJ. Functional plasticity of CH domains. FEBS Lett 2002;513:98–106.10.1016/S0014-5793(01)03240-9 [PubMed: 11911887]
9. Ihara E, Moffat L, Ostrander J, Walsh MP, MacDonald JA. Characterization of protein kinase pathways responsible for Ca2+ sensitization in rat ileal longitudinal smooth muscle. Am J Physiol Gastrointest Liver Physiol 2007;293:G699–710.10.1152/ajpgi.00214.2007 [PubMed: 17656444]
10. Wu X, Somlyo AV, Somlyo AP. Cyclic GMP-dependent stimulation reverses G-protein-coupled inhibition of smooth muscle myosin light chain phosphate. Biochem Biophys Res Commun 1996;220:658–663.10.1006/bbrc.1996.0460 [PubMed: 8607821]
11. Kitazawa T, Masuo M, Somlyo AP. G protein-mediated inhibition of myosin light-chain phosphatase in vascular smooth muscle. Proc Natl Acad Sci USA 1991;88:9307–9310.10.1073/pnas.88.20.9307 [PubMed: 1656467]
12. MacDonald JA, Borman MA, Muranyi A, Somlyo AV, Hartshorne DJ, Haystead TAJ. Identification of the endogenous smooth muscle myosin phosphatase-associated kinase. Proc Natl Acad Sci USA 2001;98:2419–2424.10.1073/pnas.041331498 [PubMed: 11226254]
13. Wu X, Haystead TA, Nakamoto RK, Somlyo AV, Somlyo AP. Acceleration of myosin light chain dephosphorylation and relaxation of smooth muscle by telokin. Synergism with cyclic nucleotide-activated kinase. J Biol Chem 1998;273:11362–11369.10.1074/jbc.273.18.11362 [PubMed: 9556631]
14. Woodrum D, Pipkin W, Tessier D, Komalavilas P, Brophy CM. Phosphorylation of the heat shock-related protein, HSP20, mediates cyclic nucleotide-dependent relaxation. J Vasc Surg 2003;37:874–881.10.1067/mva.2003.153 [PubMed: 12663991]
15. Walker LA, MacDonald JA, Liu X, Nakamoto RK, Haystead TA, Somlyo AV, Somlyo AP. Site-specific phosphorylation and point mutations of telokin modulate its Ca2+-desensitizing effect in smooth muscle. J Biol Chem 2001;276:24519–24524.10.1074/jbc.M103560200 [PubMed: 11346659]

16. Wooldridge AA, MacDonald JA, Erdodi F, Ma C, Borman MA, Hartshorne DJ, Haystead TA. Smooth muscle phosphatase is regulated in vivo by exclusion of phosphorylation of threonine 696 of MYPT1 by phosphorylation of Serine 695 in response to cyclic nucleotides. J Biol Chem 2004;279:34496–34504.10.1074/jbc.M405957200 [PubMed: 15194681]

17. Nakamura K, Koga Y, Sakai H, Homma K, Ikebe M. cGMP-dependent relaxation of smooth muscle is coupled with the change in the phosphorylation of myosin phosphatase. Circ Res 2007;101:712–722.10.1161/CIRCRESAHA.107.153981 [PubMed: 17673671]

18. Quensel C, Kramer J, Cardoso MC, Leonhardt H. Smoothelin contains a novel actin cytoskeleton localization sequence sequence with similarity to troponin T. J Cell Biochem 2002;85:403–409.10.1002/jcb.10143 [PubMed: 11948695]

19. Niessen P, Clement S, Fontao L, Caponnier C, Teunissen B, Rensen S, van Eys G, Gabbiani G. Biochemical evidence for interaction between smoothelin and filamentous actin. Exp Cell Res 2004;292:170–178.10.1016/j.yexcr.2003.09.005 [PubMed: 14720516]

20. Zhang W, Wu Y, Du L, Tang DD, Gunst SJ. Activation of the Arp2/3 complex by N-WASp is required for actin polymerization and contraction in smooth muscle. Am J Physiol Cell Physiol 2005;288:C1145–1160.10.1152/ajpcell.00387.2004 [PubMed: 15625304]

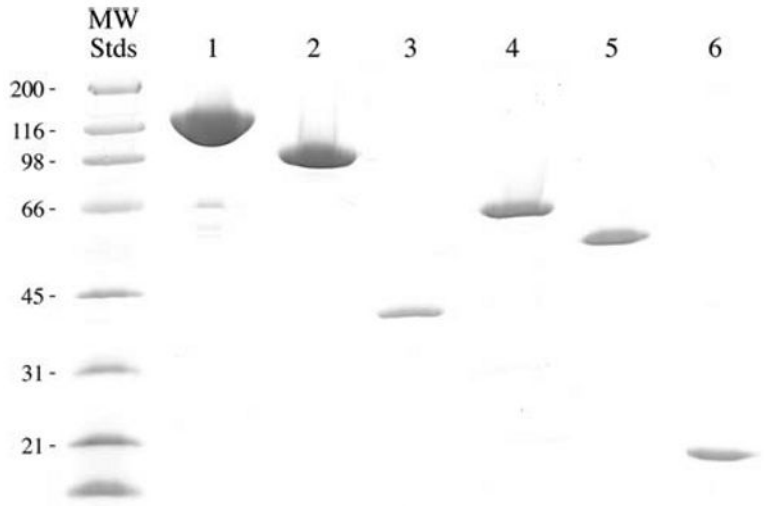

Fig. 1.

Purification of recombinant SMTNL1 proteins. Coomassie stained SDS-PAGE (10% acrylamide) gel of recombinant GST-SMTNL1 fusion proteins (GST-SMTNL1-WT, Lane 1; GST-SMTNL1-ΔCH, Lane 2; GST-SMTNL1-CH, Lane 3) and Precission Protease-cleaved SMTNL1 proteins (SMTNL1-WT, Lane 4; SMTNL1-ΔCH, Lane 5; SMTNL1-CH, Lane 6)

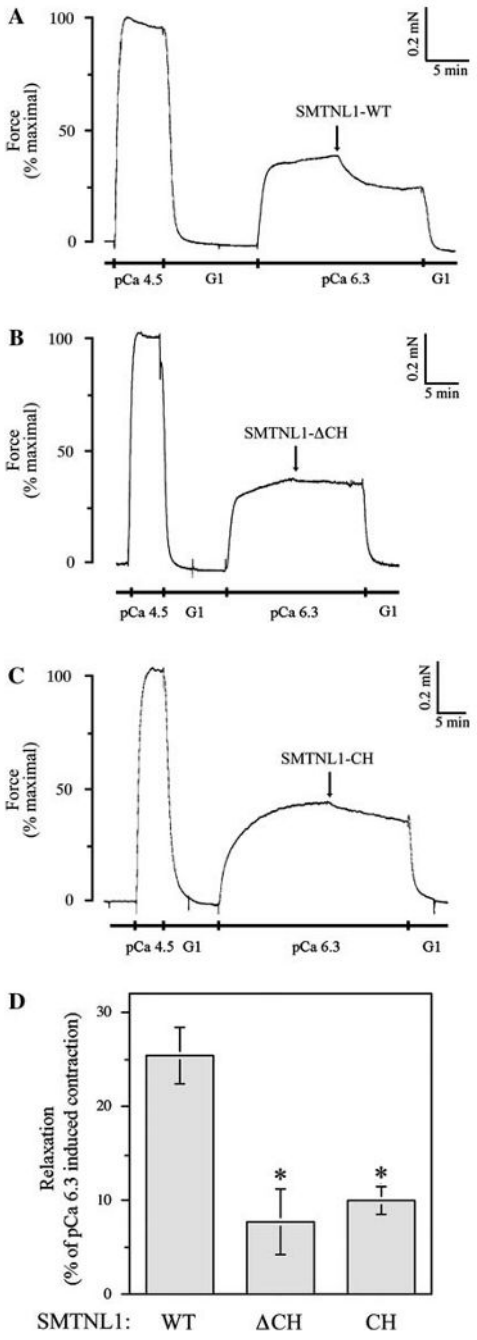

Fig. 2.

Relaxation of rabbit ileum by various SMTNL1 proteins. *β*-escin permeabilized rabbit ileum smooth muscle strips were contracted by addition of submaximal calcium solution (pCa 6.3). Relaxant responses to PreScission Protease-cleaved SMNTL1 proteins were recorded. SMTNL1-WT (**a**), SMTNL1-ΔCH (**b**) and SMTNL1-CH (**c**) proteins (10 μM) were added at the plateau of contraction. Data is representative of at least four experiments for each SMTNL1 variant. In (**d**), a summary of relaxation induced by SMTNL1 protein variants is presented. Percent relaxation is calculated from the plateau of the pCa 6.3 contraction. Data are means ± S.E.M. for $n = 4–9$ separate experiments. * Significantly different from relaxation in the presence of SMTNL1-WT protein ($P < 0.005$)

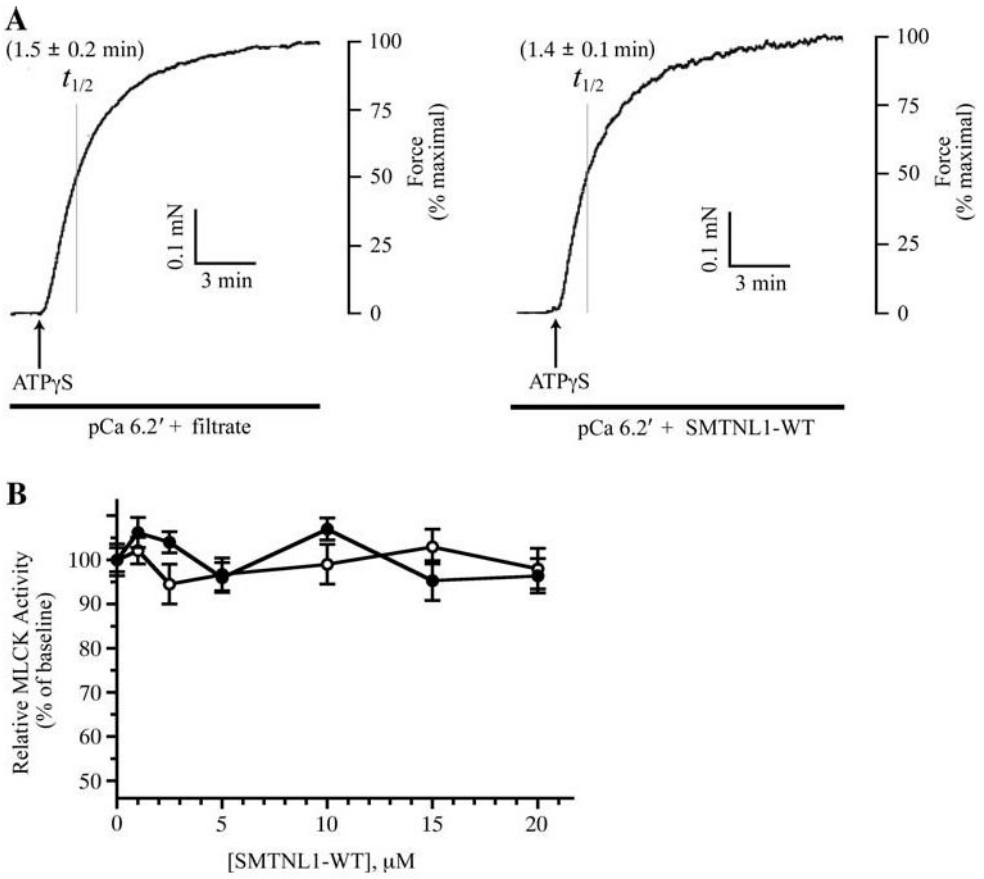

Fig. 3.

Lack of effect of SMTNL1 on MLCK activity in situ and in vitro. In (**a**), ileal smooth muscle strips (*β*-escin permeabilized) were incubated in Ca^{2+}-free, ATP-free solution at 15°C for 20 min to deplete endogenous ATP and then in ATP-free pCa6.2 solution (pCa 6.2′) in the absence (*left panel*) or presence (*right panel*) of 10 μM SMTNL1-WT. Subsequently, contraction was initiated by the addition of 2 mM ATPγS. Force traces are representative of four independent experiments for each condition. The time required to reach half-maximal force ($t_{1/2}$) was calculated from the contractile traces. Error bars indicate S. E. M., $n = 4$. In (b), the activity of purified smooth muscle MLCK (0.1 μg) toward chicken gizzard LC_{20} was assayed in the presence (●) or absence (○) of PreScission Protease-cleaved SMTNL1-WT. MLCK activity is expressed relative to baseline activity detected in the absence of SMTNL1-WT or GST protein. Error bars indicate S. E. M., $n = 4$

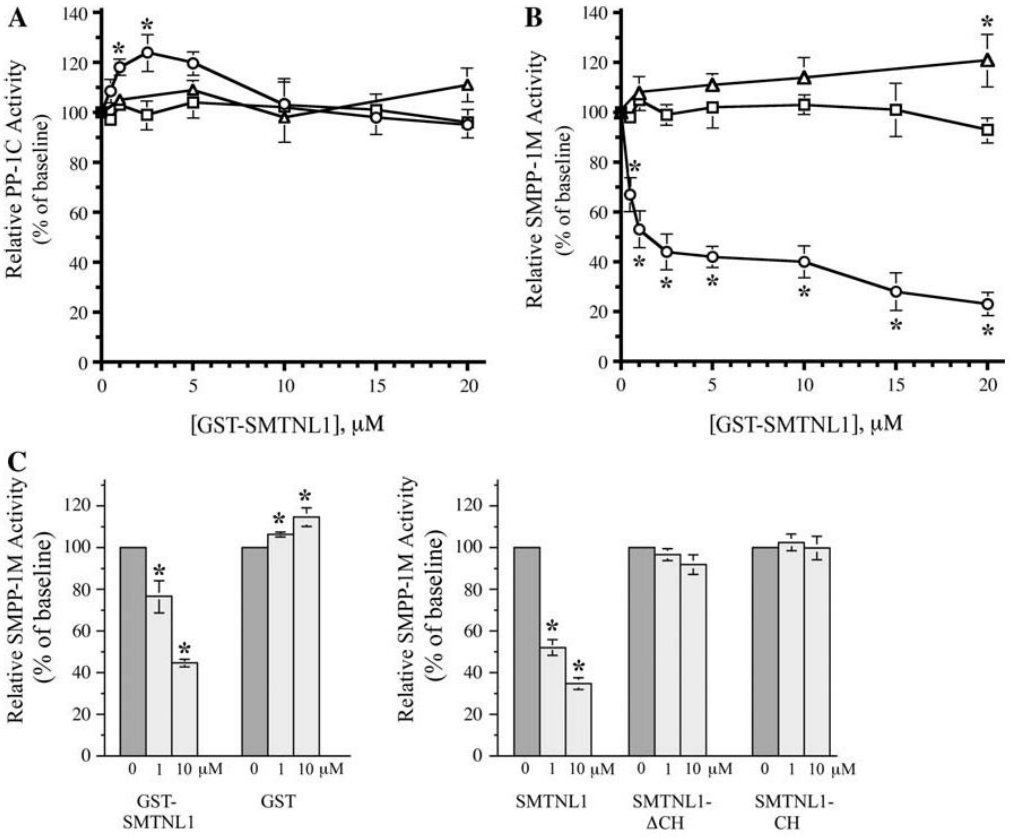

Fig. 4.

Full-length SMTNL1 protein can inhibit SMPP-1M activity in vitro. In (**a**), the effect of GST-SMTNL1-WT on PP-1C activity is shown. The activity of PP-1C (0.4 nmol) toward ^{32}P-labeled LC_{20} was measured in the presence of increasing amounts of wild-type GST-SMTNL1 (*open circles*), GST (open triangles) or filtrate (open squares). In (**b**), the activity of SMPP-1M (purified PP-1C and MYPT1 proteins; 0.4 : 1 molar mixture, respectively) toward ^{32}P-labeled LC_{20} was measured in the presence of increasing amounts of wild-type GST-SMTNL1 (*open circles*), GST (*open triangles*) or filtrate (*open squares*). In (**c**), the inhibitory effects of SMTNL1 proteins on SMPP-1M activity toward ^{32}P-labeled LC_{20} are summarized. Wild-type GST-SMTNL1 and GST (as a control) proteins as well as PreScission Protease-cleaved SMTNL1 variants (SMTNL1, SMTNL1-ΔCH and SMTNL1-CH) were used. Data are means ± S.E.M. for $n = 4$ separate experiments. * Significantly different from SMPP-1M activity in the absence of added SMTNL1 protein ($P < 0.05$). Where S.E.M. bars are not visible, they are contained within the dimensions of the symbols

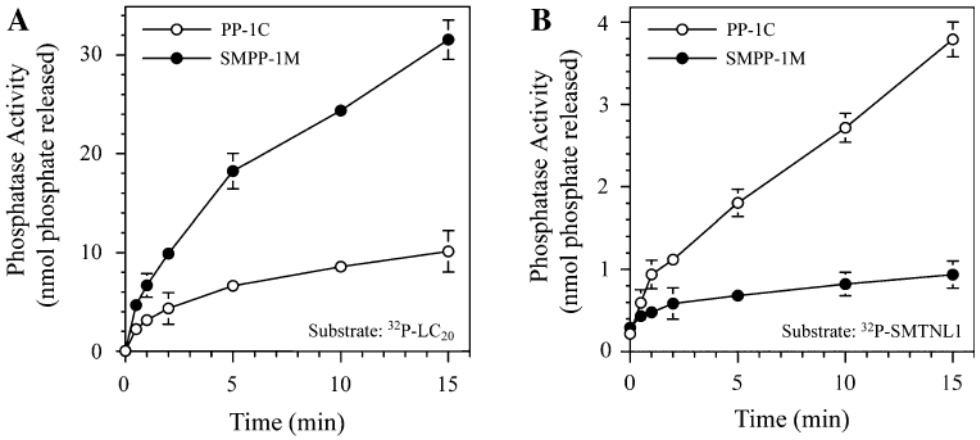

Fig. 5.

Measurement of PP-1C and SMPP-1M phosphatase activity toward ^{32}P-labeled LC_{20} and ^{32}P-labeled SMTNL1. ^{32}P-labeled LC_{20} (**a**) and ^{32}P-labeled wild-type SMTNL1 (**b**) were used as substrates for PP-1C (0.4 nmol) in the presence and absence of myosin-targeting subunit, MYPT1 (chicken M130, 1.0 nmol). Reactions were initiated by addition of ^{32}P-labeled substrate (5 μg) and terminated at the indicated time by addition of trichloroacetic acid. Cleaved ^{32}P-phosphate was measured by Cerenkov counting