Integration of signal pathways for stretch-dependent growth and differentiation in vascular smooth muscle

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STRETCH OF THE BLOOD VESSEL WALL by intraluminal pressure stimulates growth and remodeling to compensate for the increased wall tension, such that the tissue stress (force per cross-sectional area) is essentially normalized (23). Growth under these conditions occurs with smooth muscle cells maintained in a contractile phenotype, which differs from the growth pattern seen in vascular lesions, where smooth muscle cells migrate and proliferate after modulation to a synthetic phenotype (5). Although the use of isolated smooth muscle cells stretched on a flexible membrane has provided substantial insights into the mechanisms of smooth muscle cell biomechanics and phenotype regulation, the mechanisms of stretch-dependent signaling are probably not identical to those in intact tissue. The use of organ culture of vascular smooth muscle combines the three-dimensional environment of the cells with the advantages of an in vitro condition.

The portal vein is a low-pressure vessel that rapidly hypertrophies under increased transmural pressure, as shown in rat portal hypertension in vivo (26). The hypertrophy particularly affects the longitudinal muscle layer, which dominates in the portal vein, suggesting that in this intraabdominal vessel the major direction of wall stress is longitudinal. By applying longitudinal stress during organ culture of portal veins, we (48) demonstrated in vitro that stretched vessels showed greater contractility and rate of protein synthesis than control vessels kept unstretched during culture. The veins were stretched to the optimal extension for force development, approximately corresponding to the in vivo length of the vessel. The difference relative to unstretched veins therefore highlights the importance of physiological pressure for the maintenance of structural and functional integrity of the vessel wall.

Further studies (45, 46) using the portal vein model revealed that stretch causes endogenous release of angiotensin II and of endothelin-1 and promotes the synthesis of smooth muscle-specific proteins by a mechanism requiring an intact cytoskeleton. We then directly demonstrated that stretch causes Rho activation and increased actin polymerization and that this correlates with synthesis of smooth muscle-specific proteins, including α-actin, smooth muscle protein 22 (SM22), tropomyosin, desmin, and calponin (2). Furthermore, we showed that actin polymerization does indeed affect the synthesis of smooth muscle-specific proteins by using the actin filament-stabilizing agent jasplakinolide. Although the effects of stretch on the intact vascular wall are likely to be complex, these results are in line with recent findings on the role of actin polymerization for smooth muscle-specific gene expression under the control of serum response factor (SRF) in concert with tissue-specific cofactors such as myocardin and MAL/MKL1 (7, 11, 25, 30, 34, 41).

Protein synthesis is regulated by the MAPK pathway and phosphatidylinositol 3-kinase (PI3K) pathway via both eukaryotic initiation factors (13) and 70-kDa ribosomal protein S6 kinase (12). The relative roles of these two pathways in stretch-dependent protein synthesis in the intact vasculature are not well known. Depolymerization of actin filaments with latrunculin B, cytochalasin D, or Rho inhibition prevents stretch-dependent ERK1/2 phosphorylation, growth, and leucine incorporation (46), suggesting a role of actin polymerization in the regulation of global protein synthesis.

Stretch-induced regulation of signal pathways is likely to involve integrin activation. Integrins connect the cell interior to the extracellular matrix and are closely linked to the cytoskeleton. In cultured airway smooth muscle cells, mechanical stretch induces focal adhesion formation (40). Focal adhesions involve the clustering of integrin complexes as well as the recruitment and autophosphorylation of focal adhesion kinase

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(FAK) (35). Phosphorylation of FAK has been shown to regulate MAPK activity and protein synthesis in response to angiotensin II and to steady, but not pulsatile, stretch of vascular smooth muscle cells (15, 22). FAK phosphorylation has also been implicated in the regulation of other signals involved in growth and differentiation of smooth muscle, such as the PI3K and Rho pathways (6, 35). Stretch-induced FAK activation is dependent on an intact cytoskeleton, as has been shown in myocardial cells (43), while, on the other hand, stretch-induced FAK activation may itself influence the cytoskeletal organization. This suggests the actin cytoskeleton as a convergence point between the effects of stretch on growth and proliferation, on the one hand, and on specific expression of smooth muscle differentiation markers, on the other hand. We therefore hypothesized that the signal pathways mediating the effects of stretch on both the contractility and growth of smooth muscle are influenced by the cytoskeleton and would be affected by altered actin polymerization. This effect may be mediated by integrin signaling and/or a direct effect of actin on intracellular mediators. If stabilization of actin filaments has an opposite effect on trophic signaling to that of actin depolymerization, evidence for a causal relationship would be strengthened.

In this study, we used the intact mouse portal vein to investigate acute and long-term effects of stretch on MAPK, PI3K, and FAK signaling. The specific roles of these signaling pathways in stretch-dependent global and contractile protein synthesis were evaluated, and the influence of actin polymerization was studied by stabilization of actin filaments with jasplakinolide.

EXPERIMENTAL PROCEDURES

Preparation of portal veins and organ culture. Female NMRI mice (30–35 g) were killed by cervical dislocation as approved by the regional Animal Ethics Committee of Lund and Malmö Universities. The portal vein was removed and dissected free from fat and connective tissue under sterile conditions. Veins were then incubated at 37°C in DMEM-Ham’s F-12 (1:1) with 2% dialyzed FCS and 10 nM insulin as previously described (46). To stretch the vessel wall, each portal vein was loaded with a 0.3-g weight. This caused extension of the vessel wall to a passive tension of 3 mN, and equilibrated in HEPES buffer [composed of (in mM) 135.5 NaCl, 5.9 KCl, 2.5 CaCl₂, 1.2 MgCl₂, 11.6 glucose, and 11.6 HEPES; pH 7.4, 37°C] for 1 h prior to the experimental protocol. Veins were contracted with high-K⁺ HEPES buffer (80 mM NaCl exchanged for KCl). Integrated force over 5 min was evaluated, and the mean of three contractures was used for the assessment of contractile force. Following each contracture, portal veins were relaxed in normal HEPES buffer for 10 min. For measurements of spontaneous contractile activity, the mean amplitude of the two bursts of activity prior to the first high-K⁺ contracture was used. To induce maximal contractions by irreversible myosin light chain phosphorylation, 2 μM calyculin A (Sigma) was used at the end of the experimental protocol. Maximal contractile force was used in summarized data.

Confocal microscopy. Portal veins were incubated either stretched or unstretched for 24 h in organ culture with or without the addition of 100 nM jasplakinolide in the culture medium. Portal veins were then washed in ice-cold PBS (pH 7.4) followed by fixation in 4% formaldehyde in PBS for 1 h. Vessels were washed twice for 10 min in PBS, embedded in Tissue-Tek (Sakura), and frozen. Transverse sections (5 μm) were cut, permeabilized for 15 min in 0.2% Triton X-100, blocked in 2% BSA for 1 h, and stained for α-actin (mouse monoclonal anti-α-smooth muscle actin, 1:400, Sigma) in blocking buffer for 1 h. All solutions were prepared in PBS (22°C). Tissue sections were washed three times in PBS for a total of 45 min, incubated with secondary antibody (Cy5-conjugated donkey antimouse, 1:500, Jackson Immunoresearch Laboratories) for 1 h, and mounted with Aqua Poly/Mount (Polysciences) to prevent photo-bleaching. Slides were examined using a Zeiss LSM 510 confocal microscope.

Statistics. Values are presented as means ± SE. Except as stated, Student’s t-test was used for the evaluation of statistical significance. For multiple comparisons, one-way ANOVA and the Bonferroni post test was used. P < 0.05 was considered statistically significant.

RESULTS

Time course of FAK, ERK, and Akt phosphorylation. FAK phosphorylation was evaluated using a phospho-specific antibody to Tyr397. Figure 1A shows that continuous stretch by a hanging weight activated FAK biphase, with peaks at 5–15 min as well as at 24–72 h. The early stretch-induced FAK activation correlated with a rapidly increased phosphorylation of ERK1/2 in the MAPK pathway, whereas for the remainder of the time course, ERK1/2 was only slightly higher in stretched than in unstretched veins (Fig. 1B). Akt phosphorylation likewise was slightly higher in stretched versus unstretched veins, but without any clear peak value (Fig. 1C).

Roles of ERK and PI3K in total protein synthesis. Growth of the smooth muscle involves an increased overall protein synthesis. We used autoradiography to simultaneously evaluate the synthesis of specific proteins and of all proteins together (global protein synthesis). Figure 2A shows autoradiographs of gels prepared from a stretched and an unstretched portal vein together with densitometric scans. A number of bands representing contractile and cytoskeletal proteins are shown (Fig. 2A, asterisk). We have previously identified these proteins on two-dimensional gel autoradiographs and by using SM22-deficient mice (2, 47). In Fig. 2A, note particularly that SM22

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is a clearly distinguished band that is considerably weaker in the unstretched vein than in the stretched vein.

The MEK inhibitors PD-98059 (10 μM) and U-0126 (20 μM) reduced global protein synthesis in both stretched and unstretched portal veins (Fig. 2B). The reduction was relatively greater in stretched veins (PD-98059: −20 ± 3% vs. −11.5 ± 3%; U-0216: −17 ± 2% vs. −11 ± 6%), consistent with earlier findings that stretch-dependent protein synthesis involves MEK-ERK1/2 activation (3, 48). After inhibition of the MEK-ERK1/2 pathway, there was, however, still a significant effect of stretch on protein synthesis.

Inhibition of PI3K using LY-294002 (20 μM) reduced global protein synthesis in both stretched and unstretched portal veins (−32 ± 7% vs. −25 ± 2%). Thus, stretch sensitivity of the protein synthesis was maintained (Fig. 2C). Similar results were observed using the mammalian target of rapamycin (mTOR) inhibitor rapamycin (1 μM), which lowered protein synthesis by 30 ± 3% and by 23 ± 5% in stretched and unstretched portal veins, respectively. When LY-294002 and rapamycin were used in combination, an additive effect was observed, as protein synthesis was decreased by 57 ± 3% and by 41 ± 7% in stretched and unstretched portal veins, respectively.

Roles of ERK and PI3K in smooth muscle-specific protein synthesis. The synthesis of several SRF-regulated smooth muscle proteins depends strongly on stretch, as shown in Fig. 2A. This effect correlates with Rho activation and increased actin polymerization in response to stretch (2, 25, 41). However, the roles of the MAPK and PI3K pathways in the regulation of smooth muscle differentiation marker expression need to be considered as well.

Figure 3, A and B, shows synthesis rates of SM22, tropomyosin, and actin and the effects of inhibitors of the MAPK and PI3K pathways. Stretch sensitivity of smooth muscle-specific protein synthesis was maintained in the presence of these inhibitors (Fig. 3, A and B). The inhibitors of the PI3K pathway, LY-294002 and rapamycin, had no obvious effect individually on smooth muscle-specific synthesis in stretched portal veins. Their combination greatly decreased protein synthesis, although some stretch sensitivity still remained (Fig. 3A). This effect of the combined inhibitors correlated with their effect on global protein synthesis in stretched veins (Fig. 2C).

Interestingly, the two MEK inhibitors, PD-98059 and U-0126, which had similar effects on global protein synthesis (Fig. 2B), affected smooth muscle-specific synthesis differently. While PD-98059 reduced the synthesis of these proteins in stretched veins, there was no such effect of U-0126 (Fig. 3B). The probable reason for this difference is that PD-98059 was found to also inhibit phosphorylation of the actin-depolymerizing factor cofilin, while U-0126 had no such effect (Fig. 3, C and D). Cofilin phosphorylation in the portal vein is stretch dependent (2), and decreased phosphorylation is expected to cause actin depolymerization and inhibition of smooth muscle-specific protein synthesis. In unstretched veins, where protein synthesis is quite low, neither inhibitor had any effect on the synthesis rate.

Cross talk between MAPK and PI3K pathways. To evaluate the effectiveness of the different inhibitors, we performed Western blots for Akt and ERK1/2 on portal veins incubated with U-0126 and LY-294002 for 24–72 h. While U-0126 inhibited ERK1/2 phosphorylation by ~90%, LY-294002 inhibited Akt phosphorylation by ~55% in stretched portal veins. LY-294002 caused about a doubling of ERK1/2 phosphorylation (Fig. 4, A and C). This was seen in both stretched and unstretched veins, in which basal ERK1/2 activation did not differ in culture (cf., Fig. 1B). A converse effect was also seen in that inhibition of ERK phosphorylation caused a 70% increase in Akt phosphorylation at 24 h of culture in both stretched and unstretched veins (Fig. 4B). This effect was reduced at 72 h. Neither of the inhibitors affected FAK phosphorylation, and the effect of stretch on FAK phosphorylation (cf., Fig. 1A) persisted in the presence of the inhibitors (Fig. 4C).

Effects of actin filament stabilization on intracellular signaling and protein synthesis. Jasplakinolide is known to bind to actin filaments and prevent their depolymerization by cofilin, but it also enhances the rate of actin filament nucleation, causing a decrease in monomeric G-actin and the appearance of disordered polymeric F-actin (4). Consistent with these
effects, jasplakinolide caused a decrease in the F-to-G-actin ratio and increased the rate of SM22 synthesis in unstretched portal veins (2). The effect was concentration dependent, and a maximal increase in SM22 synthesis was found at a concentration of 100 nM, which was therefore used in the present experiments. Figure 5 shows \( /H9251\)-actin staining in control and jasplakinolide-treated portal veins. In the intact tissue, the effect of jasplakinolide on cellular structure and the organization of actin stress fibers was difficult to evaluate. However, we found no obvious changes in cell morphology in tissue sections examined under light microscope (data not shown). In stretched portal veins incubated with jasplakinolide, there was a slightly patchier \( /H9251\)-actin staining than in stretched controls (Fig. 5). This may be due to some aggregation of actin into amorphous masses, as previously reported (4). However, in unstretched portal veins, jasplakinolide had no apparent effect on \( \alpha\)-actin staining.

Stabilization of actin polymerization by jasplakinolide activated ERK1/2 by two- to threefold in both stretched and unstretched portal veins (Fig. 6A and B). The increase in ERK1/2 phosphorylation was sensitive to inhibition of MEK (Fig. 6B) but did not depend on FAK activation, since FAK phosphorylation was not increased by stretch in the presence of jasplakinolide (Fig. 6C). Akt phosphorylation was unaffected by jasplakinolide (Fig. 6D).

The effect of jasplakinolide on protein synthesis is shown in Fig. 7A. The presence of jasplakinolide during culture of unstretched portal veins affected protein synthesis in much the same way as culture under stretch, as seen by a comparison with Fig. 2C. Both global synthesis and the synthesis of differentiation marker proteins were increased (Fig. 7B). In contrast, jasplakinolide had no effect on synthesis rates in stretched veins, despite the increase in ERK1/2 phosphorylation (Fig. 7A).

**Effect of jasplakinolide on smooth muscle contractility.** To evaluate the contractile properties of smooth muscle after treatment with jasplakinolide, portal veins cultured for 48 h with or without jasplakinolide were mounted in organ baths for isometric force measurement. Despite the structural effects of jasplakinolide (Fig. 5), treated veins showed an essentially normal pattern of spontaneous activity (data not shown). To avoid possible effects of culture and/or jasplakinolide on activation mechanisms, veins were contracted using the cell-permeable phosphatase inhibitor calyculin A, which causes...
irreversible myosin light chain phosphorylation and maximal contraction (42). Figure 8A shows examples of calyculin A-induced contractions in portal veins cultured with or without stretch and in the presence or absence of jasplakinolide. Summarized data are shown in Fig. 8B together with results from high-K⁺ activation. Culture without stretch led to a decrease in contractility, which is in accordance with earlier results in the rat portal vein showing remodeling and atrophy in the absence of stretch (48). In the presence of jasplakinolide, contractility of stretched veins was impaired, and, interestingly, there was no effect of stretch during culture.

DISCUSSION

Stretch of the vessel wall elicits multiple signals acting on diverse intracellular cascades, and even though many of these have been elucidated in simplified experimental systems, their integration in the intact vessel is poorly understood. Here, we investigated the relationship between actin polymerization and the MAPK and PI3K pathways, which all have been shown to influence vascular protein synthesis but have differing effects on growth and differentiation. The organ culture experiments reported here were done in the presence of insulin (10 nM) and a low concentration (2%) of dialyzed FCS, a combination we have previously found to be suitable for preserving contractility and allowing either positive or negative growth regulation by factors such as angiotensin II, endothelin-1, and inhibitors of signaling pathways (45, 46).

Stretch caused a biphasic activation of FAK at 5–15 min and at 24–72 h, which correlates well with the effect of increased steady pressure in the aorta (22). This time course may reflect an initial phosphorylation due to stretch of preexisting focal adhesions, followed by a rearrangement to accommodate for the maintained mechanical load. Evidence for stretch-dependent binding of cytoskeletal proteins to focal adhesions has been obtained using Triton X-100-extracted cytoskeletons (37). Recent modeling studies on vascular smooth muscle cells, based on atomic force microscopy data, have suggested that stretched actin filaments are gradually replaced by newly formed filaments with a “homeostatic” level of stretch (32). In the present work on the portal vein, ERK1/2 was rapidly but transiently phosphorylated by stretch, while Akt phosphorylation did not show any acute response to stretch. Both ERK1/2 and Akt phosphorylation tended to be increased during maintained stretch, but this effect was small and significant increases in stretched veins were seen only occasionally during the time course from 5 min to 72 h. These subtle differences over time may be functionally important for growth regulation in the intact vascular smooth muscle subjected to physiological stretch. For example, a sustained ERK activation is required for accumulation of immediate-early genes (31). However, it is likely that the effects of continuous stretch on protein synthesis are primarily mediated by stretch-sensitive Rho activity and actin polymerization in concert with ERK1/2 and Akt activity.

PI3K signaling is known to be important in the regulation of global protein synthesis (14) and smooth muscle differentiation, although findings are partly conflicting. Results have suggested that PI3K regulates smooth muscle-specific protein expression by effects at both the transcriptional (18, 19, 27) and translational (17) levels. Recent reports have also suggested that Akt phosphorylation in vascular smooth muscle is sensitive to stretch exerted by vascular stenting (49) or exposure of a vein graft to arterial pressure and flow (38). In light of the present results, it is possible that this effect is caused by the stretch and possible injury to the smooth muscle cells associated with these interventions or to flow-induced effects mediated via the endothelium. Vascular injury and fluid shear stress are both known to increase Akt phosphorylation (10, 39). In our study, vessels were not perfused and were stretched to their optimal length for force development. In this model, the mechanical stress on the vessel wall approximates the physiological state of smooth muscle as opposed to the unstretched condition.
In the present study, we demonstrated that the stretch sensitivity of global as well as contractile protein synthesis is maintained in the presence of inhibitors of the MAPK pathway (U-0126 and PD-98059) or PI3K pathway (LY-294002 and rapamycin) when used separately. In combination, the PI3K and mTOR inhibitors greatly depressed global protein synthesis and abolished its stretch sensitivity. This demonstrates that a certain degree of activation of the PI3K signaling pathway is necessary for stretch-mediated protein synthesis, although mechanical stretch is not a major stimulus for Akt phosphorylation. Interestingly, inhibitors of the PI3K pathway, when used separately or in combination, caused a relatively greater decrease in protein synthesis than did the MEK inhibitors. This suggests that basal protein synthesis may be more influenced by the PI3K pathway than by the ERK-MAPK pathway.

In general, smooth muscle-specific protein synthesis of SM22, actin, and tropomyosin, which are easily identified as stretch-sensitive proteins on an autoradiograph, were not significantly affected by the inhibitors of PI3K or ERK-MAPK pathways (U-0126). This indicates that the contractile phenotype is maintained in stretched portal veins in the presence of inhibitors of the MAPK or PI3K pathways. One exception to this was the MEK inhibitor PD-98059, which had similar effects as U-0126 on ERK1/2 phosphorylation and global protein synthesis but also tended to decrease synthesis of all smooth muscle-specific proteins analyzed. This is likely due to the fact that PD-98059, unlike U-0126, inhibits phosphorylation of the actin-depolymerizing factor coflin, possibly by a mechanism unrelated to MEK inhibition. Decreased phosphorylation of coflin would be expected to cause depolymerization...
of actin filaments and reduced transcription of smooth muscle-specific genes. We have observed earlier that inhibition of the Rho-ROCK pathway causes decreased cofilin phosphorylation and a marked reduction in contractile protein synthesis (2, 46). Thus, effects of stretch on contractile differentiation seem to be the mainly mediated via the Rho-ROCK pathway.

We showed in this study that inhibition of PI3K/Akt increases ERK1/2 phosphorylation and that inhibition of MEK/ERK transiently promotes Akt phosphorylation. This indicates that a reciprocal cross talk exists between these pathways, which is likely to be important for the effect of such inhibition on protein synthesis and growth of vascular smooth muscle. In other cell types, phosphorylated Akt has been shown to inhibit MAPK activity in HEK-293 cells and in differentiated C2C12 myotubes via the phosphorylation and inactivation of Raf at Ser259 (36, 50). Akt phosphorylation has also been shown to negatively regulate p38 activity (16), and inhibition or silencing of glycogen synthase kinase-3 downstream of PI3K/Akt results in increased MAPK activation via a PKC-δ-dependent mechanism (44). Regulation of PI3K/Akt activity by MAPK/ERK has, to our knowledge, not been demonstrated previously, and the mechanism needs to be examined, including the dependency of the effect on inhibitor concentrations.

The dynamic state of the actin cytoskeleton involves the polymerization and depolymerization of actin filaments, which alters the ratio of filamentous F-actin to monomeric G-actin. Mechanical stretch of the portal vein, as well as the presence of jasplakinolide, results in a relative increase in the F-to-G-actin ratio (2). In the present study, we found that actin filament stabilization by jasplakinolide eliminated the effect of maintained stretch on FAK activation. Jasplakinolide also promoted global protein synthesis in unstretched veins, which is partly dependent on ERK1/2 phosphorylation. Our results indicate that stabilization of actin filaments maintains a high level of ERK1/2 activity in both stretched and unstretched portal veins. Akt phosphorylation was not affected by jasplakinolide, which again argues against the PI3K-Akt pathway as an important component in actin-linked stretch sensing.

Some previous evidence has linked actin polymerization to ERK activation. Agents that depolymerize actin filaments also inhibit ERK phosphorylation in response to stretch of vascular smooth muscle (28, 33, 46). Aikawa and co-workers (1) showed a small but significant activation of ERK after transfection of cardiac myocytes with constitutively active mutants of Rho. Furthermore, stabilization of actin filaments with jasplakinolide prevented dephosphorylation of ERK in response to actin depolymerization by nitric oxide (20). Our observations suggest that the state of actin polymerization, regardless of applied stretch, is able to regulate the activity of the MAPK cascade. The mechanism by which this occurs is still unknown, but phosphorylated ERK1/2 has been shown to bind to actin as well as the actin binding proteins α-actinin and calponin (24). This binding is likely dependent on the filamentous or monomeric state of actin and may regulate ERK1/2
activation and localization. Contractile agonists induce polymerization of actin in intact smooth muscle (8, 29) and association of ERK with actin filaments in differentiated smooth muscle cells (21). Furthermore, nuclear localization of phosphorylated ERK in vascular smooth muscle cells is increased by RhoA inhibition and disruption of the cytoskeleton (51).

Although jasplakinolide is known to promote actin polymerization, high concentrations (>200 nM) may also disrupt actin filaments of cells and induce polymerization of monomeric actin into amorphous masses (4). We observed a similar effect in isolated smooth muscle cells incubated with jasplakinolide (100 nM) for 24 h (data not shown). However, in the intact portal vein, jasplakinolide did not influence the morphology of smooth muscle, and we (2) have previously demonstrated that 100 nM jasplakinolide increases the F-to-G-actin ratio in the portal vein to a similar extent as mechanical stretch. Jasplakinolide caused some aggregation of α-actin staining, primarily in stretched portal veins, and a reduction in contractile force after culture in stretched, but not unstretched, veins. Thus, the negative effects of jasplakinolide on contractility seem to be most apparent when vessels have been cultured under stretch in the presence of the compound.

We suggest that jasplakinolide affects the functional state of actin filaments. Specifically, the effects of applied force on the cytoskeleton, including focal adhesions, are eliminated in the presence of jasplakinolide. One way to view this effect is that jasplakinolide, by stabilizing the cytoskeleton, prevents restructuring under the influence of altered mechanical load. This may explain the absence of an effect of stretch on either FAK phosphorylation or force development. The actin stabilization provided by jasplakinolide also prevents the increase in G-actin concentration that otherwise occurs in unloaded veins (2) and thus precludes the decrease in the synthesis of smooth muscle-specific proteins, including contractile proteins (e.g., Refs. 25 and 34).

It is important to note that the turnover of contractile and cytoskeletal proteins in smooth muscle is slow. We (2) have previously shown that major changes in stretch-sensitive protein synthesis are not reflected by similar changes in protein content. In the rat portal vein, we calculated that organ culture

![Figure 6](http://ajpcell.physiology.org/)

**Fig. 6.** Effect of actin stabilization on FAK, ERK, and Akt signaling. Portal veins were incubated with jasplakinolide (Jas; 100 nM) for 24 h (A and C) or 72 h (B and D) and then analyzed with Western blot analysis for FAK, ERK1/2, and Akt phosphorylation with phosphospecific antibodies. In B, portal veins were incubated with either Jas alone or Jas and the MEK inhibitor U-0126 (20 μM). Summarized results were normalized to the stretched control. n = 3–6. **P < 0.01; ***P < 0.001.

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Fig. 7. Global protein synthesis is increased by jasplakinolide in unstretched portal veins. Mouse portal veins were incubated for 72 h in organ culture with [35S]methionine present during the last 24 h. Global protein synthesis was evaluated from the whole lane of an autoradiograph. Jas and the MEK-inhibitor U-0126 were present as indicated in A. The original autoradiograph in B shows the effect of Jas on global protein synthesis and on the synthesis of a number of contractile and cytoskeletal proteins. n = 3–5. *P < 0.05 and **P < 0.01.

Fig. 8. Effects of Jas on smooth muscle contractility in the portal vein. Portal veins were incubated for 48 h with or without Jas in organ culture. Vessels were then mounted on force transducers, and active force in response to high-K⁺ solution and calyculin A was evaluated. Original recordings from calyculin A-induced contractions are presented in A. Summarized data are shown in B. n = 4–7. *P < 0.05.

for 3 days caused an increase in total protein of ~13% in stretched preparations and 4% in unstretched preparations (48). The influence of stretch on the actin filament system may in fact be more decisive than that on contractile protein synthesis in determining the remodeling and force generation of the contractile/cytoskeletal system during the limited culture times used here. To address the possibility that force development under the conditions studied here was affected by an influence on activation mechanisms, we used the phosphatase inhibitor calyculin A to produce Ca²⁺-independent myosin light chain phosphorylation. The results showed that culture without stretch caused a decreased ability of the contractile system to...
generate force, irrespective of any effects on activation mechanisms. Jasplakinolide prevented the effect of stretch on force development. This may be caused by an inhibition of actin filament remodeling, which would otherwise have adapted the smooth muscle cells to the altered mechanical stretch.

In conclusion, the effects of physiological stretch on the growth and synthesis of contractile proteins can, to a considerable degree, be reproduced by stabilization of actin filaments. This effect is not dependent on FAK phosphorylation and does not involve stretch-induced remodeling, which requires a dynamic cytoskeleton.

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