Tyrosine Kinase Inhibition Prevents Deformation-Stimulated Vascular Smooth Muscle Growth

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Abstract  The goal of this study was to determine the role of tyrosine phosphorylation in transducing deformation-stimulated vascular smooth muscle growth. Rat aorta-derived vascular smooth muscle cells were cultured on flexible silicone elastomer membranes and subjected to cyclic deformation (15 cycles per minute, deformed 2 seconds, relaxed 2 seconds). Deformation significantly increased proto-oncogene expression, [3H]thymidine incorporation, [3H]leucine incorporation, and cell number. Time course studies showed an 8-hour lag between initiation of cell deformation and onset of [3H]thymidine incorporation, with peak levels achieved after 18 to 24 hours. Western analysis of protein blots from deformed cells (10 minutes) demonstrated increased levels of phosphotyrosine-containing proteins having molecular weights of 110 to 130 and 70 to 80 kD. Deformation-stimulated tyrosine phosphorylation was prevented by the tyrosine kinase inhibitor Herbimycin A. Tyrosine kinase inhibition also prevented deformation-stimulated vascular smooth muscle cell growth as measured by [3H]thymidine incorporation. Cyclic deformation stimulates vascular smooth muscle proliferation through activation of tyrosine kinases. Inhibition of tyrosine phosphorylation is an effective means of preventing deformation-induced vascular smooth muscle growth in vitro. (Hypertension. 1994;24:706-713.)

Key Words: • muscle, smooth, vascular • protein-tyrosine kinase • phosphorylation • proto-oncogenes

Proliferation of vascular smooth muscle cells is a fundamental response to vascular injury and is important in the pathogenesis of hypertensive vascular disease, atherosclerosis, and restenosis after balloon angioplasty.1,2 The individual roles of platelet- and macrophage-derived proliferative agents, vasoconstrictor agonists, and mechanical injury in modulating vascular smooth muscle proliferation are not clearly established. Given the well-known effects of mechanical load to cause hypertrophy of skeletal and cardiac muscle in vivo and in vitro,3-5 it was reasonable to suppose that mechanical factors, independent of exogenous biochemical growth promoters, could modulate the growth of vascular smooth muscle.

Until recently, the evidence implicating active or passive mechanical stress in vascular smooth muscle growth was indirect, and the results of some studies were conflicting. Supporting a role for mechanical strain as a vascular growth stimulus are models of acute or chronic hypertension in rats that develop proliferation or hypertrophy of aortic smooth muscle.6-8 A growth-promoting effect of mechanical stress has also been demonstrated in balloon-injured rat and rabbit arteries in which platelets stimulated cell migration without proliferation, implying that smooth muscle proliferation after balloon injury was independent of platelet-derived growth promoters and was a direct consequence of vascular distension.9 Finally, inhibition of exogenous growth factors with neutralizing antibodies suppresses but does not prevent balloon-induced accumulation of vascular smooth muscle cells.10,11 Therefore, as Clowes et al12 suggested, mechanical vascular distension may directly stimulate smooth muscle proliferation. However, early studies that attempted to demonstrate a proliferative effect of mechanical stress on cultured vascular smooth muscle cells suggested that cyclic deformation, while increasing synthesis of some proteins, does not promote cellular proliferation.13-15 In contrast, Wilson et al15 recently demonstrated that neonatal vascular smooth muscle cells subjected to cyclic deformation underwent hyperplasia mediated by autocrine platelet-derived growth factor (PDGF) release.

In the current study, the effects of cyclic deformation on the growth of cultured adult rat aorta-derived vascular smooth muscle cells were characterized, and the effect of interfering with tyrosine phosphorylation in those cells was examined. Our results indicate that cyclic deformation--induced vascular smooth muscle growth is mediated by tyrosine kinase activation. Tyrosine kinase inhibition is an effective means of preventing deformation-induced vascular smooth muscle growth.

Methods

Vascular Smooth Muscle Cell Culture

 Cultures of rat aorta smooth muscle cells were obtained as previously described by outgrowth of explants from thoracic aortas of 250-g male Sprague-Dawley rats.16,17 Cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% calf serum and 1% antibiotic/antimycotic solution (GIBCO BRL). Cultures were initially passaged 7 days after explantation and passaged at a 1:4 ratio twice a week thereafter. In the current studies, all experiments were performed on cells (passages 3 through 5) that had been growth arrested by 48-hour culture in DMEM plus 0.5% calf serum.

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and 1% antibiotics. All experiments were performed in serum-free DMEM supplemented with 10 μg/mL insulin and transferrin. Because streptomycin is thought to inhibit stretch-activated ion channels in some tissues, all experiments were performed in the absence of antibiotics. The smooth muscle nature of the aortic cells was confirmed by immunofluorescence staining with anti-smooth muscle actin (Sigma Chemical Co); visualized with horseradish peroxidase, and was always greater than 98%.

Cyclic deformation of the vascular smooth muscle cells was accomplished with the Flexercell unit (Flexcell Corp), which was described previously. This apparatus consists of a vacuum unit connected to a computer-controlled regulator solenoid. Cells were plated at a density of 10⁴ cells/cm² on collagen-coated (proprietary method of Flexcell, Inc) silicone elastomer-bottomed six-well culture plates (Flexcell Flex II dishes) that could be deformed by application of a vacuum. Cyclic deformations (~20 kPa) were applied for varying time durations at 15 cycles per minute (2 seconds of deformation, 2 seconds of relaxation), resulting in a maximal increase to 124% of resting length. To ensure that this protocol of cyclic deformation did not have any deleterious effects on the cultured vascular smooth muscle cells, a number of preliminary experiments were performed. Cyclic deformation for periods ranging from 1 to 24 hours did not affect vascular smooth muscle cell membrane integrity as assessed by staining with trypan blue or release of lactate dehydrogenase (assayed with a Sigma kit). Vascular smooth muscle cells remained attached to the collagen-coated silicone elastomer substrate for periods of cyclic deformation up to 96 hours. Finally, cyclic deformations up to 96 hours did not alter the pattern or degree of anti-smooth muscle actin immunofluorescent staining, thus indicating that dedifferentiation of vascular smooth muscle cells or overgrowth by fibroblasts did not occur.

**Determination of DNA and Protein Synthesis**

DNA synthesis was measured by incorporation of [³H]thymidine as previously described. Briefly, the media from quiescent vascular smooth muscle cells grown in silicone elastomer-bottomed six-well dishes was aspirated and replaced with fresh serum-free DMEM containing 1 μCi/mL of [³H]thymidine (6.7 Ci/mmol per liter, New England Nuclear) for various times as indicated. After the cyclic deformation protocol, the media was aspirated, the cells were washed rapidly with ice-cold phosphate-buffered saline (PBS), and 10% ice-cold trichloroacetic acid was added. Acid-precipitable material was scraped from the wells, vortexed, vacuum filtered over Whatman GF/C glass-fiber filters, and rinsed three times with 5% trichloroacetic acid/70% ethanol. Radioactivity trapped by the filters was determined by liquid scintillation spectroscopy.

An identical method was used for assessment of protein synthesis, except that 1 μCi/mL of [³H]leucine was substituted for [³H]thymidine.
Measurement of Proto-oncogene Expression

Vascular smooth muscle cells underwent cyclic deformation for various times as indicated. Total RNA was extracted by the method of Chomczynski and Sacchi,22 size fractionated (25 μg per lane) by electrophoresis on 1% agarose gels containing 3% formaldehyde, and blotted onto nylon membranes (Hybond N+, Amersham) by vacuum transfer. Blots were hybridized overnight at 42°C in 50% formamide, 6X SSPE, 0.5% sodium dodecyl sulfate (SDS), and 100 μg/mL salmon sperm DNA. Blots were washed at 60°C in 0.1X SSC/0.5% SDS for 1 hour and were exposed on x-ray film at ~70°C using an intensifying screen. cDNA probes used were the 1-kb Pst I-Pvu II fragment of v-fos and 1.5-kb Pst I fragment of v-myc23,24 labeled with 32P by random priming to a specific activity of 108 disintegrations per minute per microgram using an Amphen kit. Hybridization to β-actin mRNA served as a control for the integrity of the RNA and equal loading of lanes.

Measurement of Arachidonic Acid Metabolites

Quiescent vascular smooth muscle cells were loaded with [3H]arachidonic acid (0.5 μCi/mL) for 24 hours. After loading, the cells were washed three times in sterile PBS before addition of serum-free DMEM without antibiotics for 1 hour. Cyclic deformation was performed from 15 minutes to 24 hours. The media was removed, acidified with formic acid, extracted three times with ethyl acetate, and dried under nitrogen. Prostaglandin B2 was added to determine extraction efficiency, which averaged 80%. Arachidonic acid metabolites were resolved by reversed-phase high-performance liquid chromatography (HPLC) on an Altex ultrasphere 3-μm octadecylsil column as previously described.23 Identification of radioactive peaks was by comparison with nonradioactive external standards with prostaglandin B2 as an internal standard.

Measurement of myo-Inositol Metabolites

The methods for measuring inositol polyphosphates in cultured vascular smooth muscle cells have been described in detail.21,22 Briefly, after culture for 4 days in inositol-free medium, quiescent vascular smooth muscle cells were labeled with 10 μCi/mL [3H]myo-inositol for 24 hours. Cells were washed twice with PBS and placed in serum-free, antibiotic-free DMEM plus 10 mmol/L lithium chloride (added to inhibit hydrolysis of inositol phosphate). Cyclic deformation was performed for increasing times from 1 to 60 minutes. The medium was replaced with ice-cold 10% trichloroacetic acid. The precipitated protein was scraped from the wells and extracted with chloroform/ethanol. [3H]Inositol phosphate and polyphosphates were resolved by anion-exchange HPLC over a Whatman Partisil 10 SAX column, and quantified by use of an on-line radiation detector. Radioactive products were identified by coelution with authentic nonradioactive inositol polyphosphates as external standards. AMP, ADP, and ATP were added as internal standards and monitored by absorption at 254 nm.

Measurement of Protein Phosphotyrosine Content

Deformation-stimulated phosphorylation of protein tyrosine residues was measured by immunoblot analysis. Vascular smooth muscle cells underwent cyclic deformation for increasing times and were washed twice with ice-cold PBS. The cells were lysed at 4°C by addition of 1 mL lysis buffer (90 mmol/L NaCl, 50 mmol/L Na2HPO4, 2 mmol/L L-iodoacetic acid, 5 mmol/L ZnCl2, 20 μmol/L Tris [pH 8.0], 1% nonidet P-40, 10% glycerol, 1 mmol/L phenylmethylsulfonyl fluoride, 0.15 U/mL aprotonin, 1 mmol/L sodium orthovanadate) to each well with gentle shaking for 30 minutes. The lysates were centrifuged in a microcentrifuge (12 000 rpm for 10 minutes at 4°C), protein concentration of the supernatants was determined with the Bradford assay,22 and the samples were stored at -70°C.

SDS–polyacrylamide gel electrophoresis over 10% discontinuous acrylamide gels was performed according to the method of Laemmli25 using 50 μg protein per sample well on a minigel apparatus. Proteins were electrophoretically transferred to 0.2-μm nitrocellulose membranes, and tyrosine-phosphorylated proteins were identified by immunoblot analysis using a polyclonal anti-phosphotyrosine antibody (obtained from Gibco). Tyrosine-phosphorylated proteins were visualized with biotinylated goat anti-rabbit IgG and developed using an amplified alkaline phosphatase kit (Bio-Rad). Equivalent protein loading in gel lanes was always confirmed by Coomassie blue staining of the gels after electrophoretic transfer. The stained gel revealed residual bands of high molecular weight (>200 000 D), which allowed measurement of protein loading.

Statistical Analysis

Values are presented as mean±SEM. Student's t test (two-tailed) was used for analysis of paired and unpaired means. For comparisons of multiple means, a one-way ANOVA was used, followed by the Neuman-Keuls test. For analysis of [3H]thymidine incorporation, values were normalized to the individual (nondeformed) controls for each experiment. These data are reported as a percentage of control, and the respective control values are given in the text as counts per minute per tissue culture well. A value of P<.05 was considered significant.

Results

Effects of Cyclic Deformation on Cell Morphology and Differentiation

Because of geometric constraints inherent in the Flexcell device and previously described in detail,15,20 the character of mechanical stress applied to adherent cells varies with the position of the cell in the culture well. For instance, cells in the exact center of the well tend to be compressed, while cells plated toward the periphery will experience "stretch." Therefore, we examined the morphology and differential state of vascular smooth muscle cells at different positions within the culture well. Fig 1 shows photomicrographs of vascular smooth muscle cells, stained with anti-smooth muscle actin, at the center (left) or periphery (right) of the deformable silicone elastomer well bottom after 48 hours of cyclic stress. Cells deformed in the center of a well demonstrated a random orientation identical to that observed in nonstressed cells. In contrast, cells adherent to the well periphery tended to be oriented so that the long axis of the cell was perpendicular to the radius of the culture well. Because stretch was primarily radial at the well edges, the cells oriented to minimize applied stress. No difference in the pattern or degree of α-actin staining was apparent in cells plated at the center or periphery of culture wells.

DNA and Protein Synthesis and Cell Proliferation

Incorporation of thymidine into DNA occurs during cell cycle progression in proliferating cells and may also occur during hypertrophy of vascular smooth muscle cells showing increases in cell ploidy.1 Cyclic deformation at 0.25 Hz for 24 hours increased incorporation of [3H]thymidine into quiescent rat aortic cells to 217±30% of identical but unstretched controls (P=.001, n=14; Fig 2, left). For determination of the time course of deformation-induced DNA synthesis, cells were pulse labeled by 4-hour exposure to [3H]thymidine after increasing periods of cyclic deformation. [3H]Thymidine counts were significantly increased over baseline after 8 hours of stretch and plateaued by 18 to 24 hours (Fig 2, right).
Thus, measurable synthesis of DNA followed initiation of cell deformation by approximately 8 hours.

Increased synthesis of cellular proteins occurs during both hyperplasia and hypertrophy. Deformation-stimulated protein synthesis was measured by assaying incorporation of [3H]leucine. After 24 hours of cyclic deformation, vascular smooth muscle cell labeling with [3H]leucine increased to 123% of nonstretched controls (Fig 3). When followed for 48 hours, [3H]leucine incorporation continued to increase to 195% of control (Fig 3).

Because both hyperplasia (ie, proliferation of cells) and hypertrophy (ie, cellular enlargement without cytokinesis) can be associated with DNA and protein synthesis, we examined whether the growth response to cyclic deformation was hyperplasia or hypertrophy by comparing the increase in cell number in stretched versus nonstretched cells. Following cyclic deformation for 48 hours, cell number (1.78±0.14×10^5 cells per well) was 33% higher than control (1.32±0.09×10^5 cells per well, n=5, P=.02). This deformation-induced proliferation was consistently observed but was smaller than the response observed in identically prepared quiescent cells exposed to 10% fetal serum that demonstrated a 188% increase in cell number (3.8±0.26×10^5 cells per well) over the same time period compared with serum-deprived controls. Therefore, cyclic deformation, in the absence of growth factors, was sufficient to stimulate a proliferative response that was approximately one fifth as great as that induced by 10% serum.

**Proto-oncogene Expression**

A pattern of early cell cycle gene expression characterizes transition from the G1 to S phase of cell growth. In vascular smooth muscle cells undergoing cyclic cell stretch, we examined the time course of expression of two proto-oncogenes, c-fos and c-myc, previously shown to be expressed in vascular smooth muscle cells exposed to various biological growth factors. Fig 4 shows Northern analysis of c-fos and c-myc mRNA transcripts in vascular smooth muscle cells undergoing cyclic cell deformation for periods from 0.5 to 4 hours. The c-fos transcripts, which were not detectable in unstimulated cells (zero time point), rapidly increased by 30 minutes, peaked at 1 hour, and declined to nondetectable levels by 2 hours. In contrast, c-myc transcripts, which were minimally detected in unstimulated cells, had a more prolonged time course of increased expression lasting up to 4 hours. These studies demonstrated that the pattern of early cell cycle gene expression in mechanically deformed cells is similar to that observed in cells exposed to biological growth factors.

**Duration of Stretching (hours)**

![Graph showing DNA synthesis](image)

**Fig 2.** Graphs show deformation-stimulated vascular smooth muscle DNA synthesis. Left, Cyclic deformation at 0.25 Hz for 24 hours increased [3H]thymidine incorporation into vascular smooth muscle cells more than twofold compared with identically treated nonstretched controls (P=.001) (control [3H]thymidine incorporation averaged 5415±1098 cpm per well). Each bar represents mean±SEM of 14 experiments performed with duplicate determinations. Each separate experiment was normalized to its respective control. Right, Time course of DNA synthesis after initiation of cyclic deformation was determined by pulse labeling with [3H]thymidine at different time intervals. There was a delay of 8 hours between initiation of cyclic deformation and significant [3H]thymidine accumulation. Each point is mean±SEM of three experiments performed in duplicate.

**Fig 3.** Bar graph shows deformation-stimulated vascular smooth muscle protein synthesis. Cyclic deformation at 0.25 Hz for 24 and 48 hours increased cellular [3H]leucine accumulation. Each bar represents mean±SEM of three experiments performed with duplicate determinations. *P<.05, #P<.001 compared with respective controls.

**Fig 4.** Northern blots show analysis of c-fos and c-myc mRNA expression in vascular smooth muscle cells undergoing cyclic deformation. Total RNA was extracted from growth-arrested cells after increasing periods of cyclic deformation at 0.25 Hz. Twenty-five micrograms of RNA per lane was subjected to formaldehyde-agarose electrophoresis and Northern analysis. Blots were sequentially probed with cDNAs for c-fos (24-hour exposure), c-myc (72-hour exposure), and β-actin (24-hour exposure) (representative of three separate experiments).
Cell Signaling Assays

Cell signaling events responsible for deformation-induced vascular smooth muscle proliferation were defined in studies of phospholipase A₂, phospholipase C, and protein tyrosine kinase activities.

Phosphorylation of tyrosine residues by protein kinases follows stimulation of vascular smooth muscle cells with peptide growth factors including basic fibroblast growth factor and PDGF. The effects of cyclic cell deformation on tyrosine kinase activity were determined by Western blot analysis of phosphorytrosine-containing proteins. Preliminary experiments showed that the maximal increase in phosphorytrosine content was observed after 10 to 15 minutes of cyclic deformation (not shown). Fig 5 (left) shows the pattern of protein tyrosine phosphorylation after 10 minutes of cyclic deformation and its inhibition by the tyrosine kinase inhibitor Herbimycin A. PDGF-stimulated tyrosine phosphorylation in identically prepared cells is shown for comparison in Fig 5, right.

Because cyclic deformation activated tyrosine kinases and because tyrosine kinase activity could be prevented by addition of Herbimycin A, we examined the effects of two tyrosine kinase inhibitors, Herbimycin A and Genistein, on deformation-stimulated vascular smooth muscle cell incorporation of [³H]thymidine. When added 30 minutes before initiation of cellular deformation, both tyrosine kinase inhibitors prevented stretch-induced DNA synthesis (Fig 6). Staurosporine, a tyrosine kinase and protein kinase C inhibitor, also prevented stretch-induced thymidine uptake (Fig 6). Thus, tyrosine phosphorylation appeared to be necessary for deformation-induced cell growth.

Because thromboxane A₂ and prostaglandin F₂α have been shown to stimulate hypertrophic growth of cultured rat aortic smooth muscle cells, we examined the effects of cyclic deformation on the production of arachidonic acid metabolites. After loading with [³H]arachidonic acid, vascular smooth muscle cells underwent cyclic stretch for increasing times (1, 2, 5, 15, 30, and 60 minutes and 24 hours), and [³H]arachidonate metabolites were assayed by reversed-phase HPLC. After 15 minutes of cyclic deformation, only a small amount of labeled arachidonic acid, but no arachidonate metabolites, was detected (Fig 7). Furthermore, no arachidonate metabolites were detected during cyclic deformation for any time period up to 24 hours. In contrast, direct stimulation of cultured vascular smooth muscle cells with the calcium ionophore ionomycin (100 nmol/L) resulted in rapid formation and release of prostacyclin, ie, 6-ketoprostanand F₁α, and the long-term appearance of 5,12- and 15-HETE, in addition to mobilizing large amounts of arachidonic acid (Fig 7). Thromboxane B₂ prostaglandins F₂α, E₂, and D₂; and leukotriene B₄ were not observed after cellular stimulation by either cyclic deformation or calcium ionophore. These results suggest that phospholipase A₂ activation was not responsible for deformation-induced proliferation.

Activation of phospholipase C with production of the second messengers inositol trisphosphate and diacylglycerol is a cell signaling event common to vasoconstrictor agonists that stimulates vascular smooth muscle growth. Therefore, we examined the possibility that mechanical deformation directly activated phospholipase C by measuring phosphatidylinositol hydrolysis in [³H]myo-inositol–loaded vascular smooth muscle cells. Compared with controls, cyclic deformation for periods from 1 to 60 minutes did not increase the cellular content of [³H]inositol phosphate, bisphosphate, or either the 1,3,4- or 1,4,5-isomers of inositol trisphosphate as determined by anion-exchange HPLC (not shown). Therefore, we concluded that activation of phospholipase C did not occur during cyclic deformation and was not responsible for stretch-induced vascular smooth muscle cell proliferation.

Discussion

This study describes the role of protein tyrosine phosphorylation in transducing the direct proliferative effect of a biochemical stimulus, cyclic deformation, on cultured rat aortic smooth muscle cells. Measurements of DNA and protein synthesis, proto-oncogene expression, and cell proliferation indicated a hyperplastic response to cyclic deformation at a rate of 0.25 Hz. A necessary signal transducer for mechanical stress–induced vascular smooth muscle proliferation was phosphorylation of protein tyrosine residues. However, neither the phospholipase A₂/arachidonic acid/eicosanoid cascade nor the phospholipase C/inositol trisphosphate pathway was activated by cyclic mechanical deformation.

The observation that mechanical deformation stimulates vascular smooth muscle proliferation is consistent with the results of studies that explored the effects of mechanical stress on the growth of other muscle types. Isolated neonatal or adult cardiac myocytes respond to mechanical stretching with transient expression of c-fos[23] or increased RNA and protein synthesis but not DNA synthesis. In embryonic chicken skeletal myobutes, longitudinal stretching increases amino acid transport, amino acid incorporation into protein, and synthesis of myosin heavy chains. Initial reports of the effects of cyclic deformation on cultured vascular smooth muscle cells suggested that collagen synthesis was stimulated but proliferation was inhibited. However, the current report and two recent studies of cyclic deformation—
Fig. 6. Bar graph shows inhibition of tyrosine phosphorylation preventing stretch-stimulated DNA synthesis. Vascular smooth muscle cells were subjected to 24 hours of cyclic deformation in the presence or absence of 5 μmol/L Herbimycin A (H), 25 μg/mL Genistein (G), or 100 nmol/L staurosporine (S). All three kinase inhibitors prevented stretch-stimulated DNA synthesis.

induced growth of a cell line derived from neonatal vascular smooth muscle cells demonstrate strikingly similar increases in cell number (33% to 40%) and DNA synthesis (270% to 277%) after cyclic deformation.

Activation of one or more tyrosine kinases has been shown to transduce the mitogenic response for both protein growth factors and phospholipase C-linked mitogenic peptides such as angiotensin II, endothelin, and thrombin. We found that cyclic deformation stimulated tyrosine phosphorylation of several proteins in vascular smooth muscle cells; among these were proteins with molecular weights of 110 to 130 and 70 to 80 kD. Although the identity of these phosphoproteins is unknown, their molecular sizes approximately correspond to those of GTPase activating protein (120 kD) and p74, a cytoplasmic serine-threonine kinase.

It is not possible from these results to determine precisely how cell stress results in tyrosine phosphorylation. Prior studies of thrombin-induced vascular smooth muscle or fibroblast proliferation have demonstrated cellular synthesis of PDGF-A chain. Similarly, Wilson et al demonstrated an increase in PDGF secretion by neonatal vascular smooth muscle cells subjected to cyclic deformation and partial inhibition of deformation-induced growth by antibodies against PDGF. Thus, it is likely that mechanical stress stimulates formation of one or more endogenous growth factors that stimulate growth factor receptor tyrosine kinases. However, we noted differences in the pattern of tyrosine phosphorylation by cyclic deformation and exogenous PDGF. It is therefore possible that mechanical stress also activates cytosolic tyrosine kinases by a mechanism that is independent of growth factor receptors.

The possibility that cyclic deformation caused endogenous synthesis of other bioactive compounds that transduced the growth response was examined. Vascular tissues synthesize and release a variety of arachidonic acid metabolites when stimulated, and some eicosanoids have mitogenic potential. However, at a deformation rate of 15 cycles per second, the sole labeled product detected by reversed-phase HPLC was arachidonic acid. Furthermore, the cyclooxygenase inhibitor indomethacin is without effects on deformation-stimulated growth (not shown). Although it is possible that arachidonic acid may have direct effects on vascular smooth muscle

Fig. 7. Chromatograms show measurement of arachidonate metabolites in vascular smooth muscle cells undergoing cyclic deformation. Representative reversed-phase high-performance liquid chromatograms of culture media from cells undergoing cyclic deformation (top tracings) or stimulated with ionomycin (bottom tracings) for 15 minutes (left tracings) or 24 hours (right tracings). No arachidonate metabolites were detected in stretched cells at any time point. Ionomycin stimulated release of prostacyclin (measured as its stable hydrolysis product 6-prostaglandin F₃α) and 5, 12, and 15 HETE.
growth, it does not appear that cyclooxygenase or lipoxygenase metabolites of arachidonic acid play a role.

Phospholipase C is a common signal transducer for mitogenic vasoconstrictors, and phospholipase C-γ is also activated by epidermal growth factors and PDGF. Although there is evidence that cyclic stretch can stimulate phospholipase C-mediated production of inositol phosphates in cultured endothelial cells, we did not detect an increase in [3H]inositol phosphate or polyphosphates in cultured vascular smooth muscle cells undergoing cyclic mechanical deformation. These findings are in agreement with those of Kulik et al., who reported that static stretch but not cyclic stretch increased inositol trisphosphate and inositol tetrakisphosphate in cultured rat pulmonary vascular smooth muscle cells. Thus, the proliferative effects of mechanical stress on vascular smooth muscle do not appear to be linked to phospholipase C.

Establishing that mechanical stress stimulates vascular smooth muscle proliferation independent of the presence of exogenous growth factors has widespread clinical implications. Intravascular procedures such as balloon angioplasty, in which stretching of vessels is inherent in the performance of the procedure, may stimulate vascular smooth muscle proliferation despite interventions designed to minimize attachment of platelets or release of platelet-derived mitogens. Stress-induced vascular proliferation may also play a role in aortic medial hypertrophy, which is observed in some chronically hypertensive patients and oblitative pulmonary vascular disease associated with primary pulmonary hypertension. Future studies designed to interfere with tyrosine kinase activation in deformation-induced vascular growth have the potential to result in novel approaches for modulating vascular smooth muscle proliferation in these diseases.

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