Restoration of Resting Sarcomere Length After Uniaxial Static Strain Is Regulated by Protein Kinase Cε and Focal Adhesion Kinase

Haytham Mansour, Pieter P. de Tombe, Allen M. Samarel, Brenda Russell

Abstract—Physiological or pathological stresses and strains produce longer or wider muscle cells, but resting sarcomere length remains constant. Our goal was to investigate the cellular mechanisms for controlling this optimal, resting sarcomere length. To do so, we cultured neonatal rat cardiomyocytes on microfabricated peg-and-groove, laminin-coated silicone surfaces and applied a uniaxial static strain of 10%. Sarcomere length was accurately measured by fast Fourier transform analysis of images before, within 5 minutes of, and 4 to 6 hours after imposition of the strain. Sarcomere length of aligned cardiomyocytes (1.94±0.07 μm) was lengthened acutely (2.06±0.06 μm), and recovered (1.95±0.07 μm) by 4 hours. Puromycin, an mRNA translational inhibitor, prevented recovery of resting sarcomere length by 4 hours, thus indicating a requirement for new protein synthesis in the recovery process. Furthermore, activation of protein kinase Cε (PKCε) was necessary for length recovery, as nonselective PKC inhibitors [staurosporine (5 μmol/L) and chelerythrine chloride (10 μmol/L)], and a replication-defective adenovirus (Adv) encoding a dominant-negative mutant of PKCε prevented the restoration of sarcomere length. To assess the importance of focal adhesion complexes, cardiomyocytes were infected with an Adv encoding a dominant-negative inhibitor of focal adhesion kinase (FAK) (Adv-GFP-FRNK). Adv-GFP-FRNK also prevented resting sarcomere length recovery, whereas a control Adv encoding only GFP did not. In conclusion, using our novel culture system, we provide evidence indicating that the length remodeling process requires new protein synthesis, PKCε and FAK. (Circ Res. 2004;94:642-649.)

Key Words: microtopography ■ protein kinase C ■ signaling ■ mechanotransduction ■ remodeling

Cardiac hypertrophy is an adaptive response to increased mechanical load.1,2 Previous studies of loaded and unloaded cardiac tissue demonstrate a critical relationship between mechanical load and the regulation of myocyte remodeling.3 A constant sarcomere length is required for optimal tension development during altered loading conditions.4–9 Thus, an important outcome of cardiac remodeling is the maintenance of optimal resting sarcomere length despite changes in mechanical load leading to growth and lengthening of individual cardiomyocytes. Furthermore, the transmission of mechanical forces depends on the orientation of the cells and the function of specific cardiomyocyte cytoskeletal proteins involved in mechanochemical signal transduction. Consequently, numerous studies have examined the effect of mechanical forces on randomly oriented cardiomyocytes in cell culture.10–13 Using improved culture systems of aligned cardiomyocytes, investigators have now begun to examine the effects of the direction of strain, as well as its magnitude, on cell signaling and growth.12,14

Recently, the focal adhesion complex has been shown to be a key mediator of cardiomyocyte mechanochemical signal transduction. Focal adhesion kinase (FAK), a nonreceptor protein tyrosine kinase that localizes to focal adhesion complexes in response to cyclic stretch,15,16 has been shown to be intricately involved in converting mechanical signals into biochemical signals that regulate cardiomyocyte growth and survival. Other structural and functional proteins within the focal adhesion complex are also critically important for stretch-induced cellular remodeling.17 However, the cooperative association of integrins, the focal adhesion complex, and the actin cytoskeleton suggests an important role for FAK in the regulation of sarcomere length during periods of cardiomyocyte length remodeling.16,18,19

Recent work demonstrates that the mechanical stretch sensor also involves proteins associated with the Z disc, such as protein kinase C (PKC),20,21 On activation, the PKCε isoform translocates to the Z disc and regulates myofilament activity. PKC has also been widely studied in relation to cardiac hypertrophy.22,23 In addition, results indicating that PKCε activation can lead to the tyrosine autophosphorylation and activation of FAK24 have provided further evidence that PKC might be critical to the cardiomyocyte stretch response.

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Therefore, we explored the mediation role of PKCe between the Z-disc region and the focal adhesion complex in sarcomere length remodeling.

To explore the signaling mechanisms responsible for cardiac remodeling, our group has developed a 3-dimensional (3-D) culture system of aligned, well-attached cardiomyocytes. Culturing neonatal rat cardiomyocytes on this 3-D surface provides an environment for uniform application of mechanical strain to all cells. This system also permits cells to be suddenly stretched to study how myocyte length remodeling is regulated, and what intracellular processes are necessary for the recovery of resting sarcomere length. By using a combination of pharmacological and molecular approaches, we provide evidence for a critical role PKCe and FAK in the restoration of resting sarcomere length after uniaxial static strain.

Materials and Methods

Cell Culture
Animal experiments were performed according to Institutional Animal Care and Use Committee and NIH guidelines. Cardiomyocytes were isolated from neonatal Sprague-Dawley rats (Harlan, Indianapolis, Ind) as previously described. Briefly, hearts were removed from 1- to 2-day-old rats. Isolated ventricular myocytes were plated on laminin (25 μg/mL)-coated silicone membranes (200 000 cells/cm²) and maintained in complete medium (Dulbecco’s modified Eagle’s medium/ Nutrient Mixture F-12 HAM without L-glutamine) solution (10 mg/L) and linoleic (0.84 mg/L) fatty acids, penicillin G/streptomycin (Sigma), standard amino acid concentrations plus palmitic (2.56 mg/L) fatty acids, penicillin G/streptomycin solution (10 μg/mL), and gentamicin (50 mg/L) with 5% fetal bovine serum for 48 hours before strain experiments. Cytosine-β-D-arabinofuranoside (5 μg/mL) was added to prevent fibroblast proliferation.

Microfabrication of Textured Surface
Textured substrates for cell culture were fabricated as previously described. Briefly, the parylene-textured surface, as optically imaged in Figure 1A, consisted of vertical pegs spaced 100 μm from center to center. Groove depth was 5 μm and spacing between grooves was 10 μm at the top and bottom surface. Peg height and diameter was 10 μm. The textured side of the parylene template was then embedded into unpolymerized silicone (Dupont) and a 40 g weight was applied for 15 seconds to equally distribute the silicone beneath the template. The silicone membrane was cured (48 hours, 25°C), then cleaned with 11 mol/L hydrochloric acid (1 hour), rinsed with distilled water, and dried at 37°C overnight. Membranes were then cut into a rectangle (6×3 cm), loaded onto uniaxial static strain devices, and coated with laminin (25 μg/mL) dissolved in Dulbecco’s Modified Eagle’s Medium (Sigma) for 2 hours before cell plating.

Mechanical Strain
A strain was applied by manually turning two adjustable screws at the free end of the strain device (Figure 1B). A strain of approximately 10% was applied to longitudinally aligned cardiomyocytes in all experiments. The actual longitudinal strain applied to the membrane was determined using the micropegs on the textured surface as internal markers of length change of the membrane. When longitudinal strain was applied a small transverse compression occurred and was also measured. Uniformity of mechanical strain across the membrane was determined. Changes to substrate length were determined either parallel, or perpendicular, to the long axis of strain; each representing either longitudinal strain or transverse compression, respectively.

All myofibrillar images were captured after gluing (4 hours, 25°C) the silicone substrate to a glass slide with silicone glue (NuSil Med

Figure 1. Strain determination of substrata. A, Computer-generated 3-D output of microtopography. B, Diagram illustrating static stretch device. Five representative regions (a, b, c, d, and e) were chosen to determine regional variation of strain and compression inherent in elastic material. C, Strain and compression were assessed immediately after 10% strain (●) and after attachment of strained membrane to glass slide (○) for sarcomere length measurement. Strain was determined as the change in length parallel to axis of strain. There were no significant differences among sarcomere length values from the 5 representative regions, before or after attachment to slide. D, Similarly, compression was determined as change in length perpendicular to axis of strain. Compression of membrane was uniform immediately after strain and after attachment to membrane (n=3 independent experiments).

Myofibrillar Orientation
Orientation was determined as previously described. Briefly, cardiomyocytes grown on textured and nontextured surfaces were
photographed using a Nikon phase-contrast microscope (×20 objective). Parallel and perpendicular lined grids of known dimensions were superimposed over the images to determine cellular and myofibrillar orientation along the direction of strain. Orientation was determined both before and immediately after a 10% strain was applied to the textured and untextured (flat) surfaces.

Immunohistochemistry
At various time points (5 minutes to 48 hours) after the imposition of static strain, cells were washed in phosphate-buffered saline (PBS), fixed [4% paraformaldehyde (Fisher); 10 minutes], and washed (70% ethanol). Filamentous actin was stained with rhodamine-phalloidin (Molecular Probes) diluted in PBS (1:1000). Each membrane was incubated with 100 μL of rhodamine-phalloidin for 50 minutes and then rinsed three times (5 minutes) in PBS. Before applying a cover slip, Vectashield mounting medium (Vector Laboratories) was applied to the surface of the silicone membrane to help preserve the stain. The mounting medium contained DAPI to stain nuclei. Adv-GFP- and Adv-GFP-FRNK–infected cells were viewed under a laser scanning confocal microscope (Zeiss LSM510).

Sarcomere Length Measurement
Cardiomyocyte images, stained for actin (rhodamine-phalloidin), were captured using a Nikon epifluorescent microscope (×40 objective) with a Spot RT Color camera (Diagnostic Instruments, Inc.). Cells were selected based on sharpness of image and the number of continuous sarcomeric units in one myofibril. Images were converted to black and white and oriented in the horizontal direction. Sarcomere length measurements were acquired using computer-assisted software.29 Briefly, a region of the image encompassing 10 to 20 sarcomeres along a myofibril was selected, and each longitudinal pixel line was transformed by fast Fourier transformation (FFT) into a spatial frequency domain. The power spectra were averaged, and the spatial frequency at peak power of the first-order harmonic in the spatial frequency domain was determined. This spatial frequency was then converted into median sarcomere length (SL) across the region. The system was calibrated with glass gratings of known spacing.

Protein Synthesis Determination
To determine the effect of inhibition of de novo protein synthesis on sarcomere length recovery, cardiomyocytes were cultured onto peg-and-groove textured membranes and treated with puromycin (10 μmol/L, Sigma) for 30 minutes and 24 hours before application of strain. Myocytes remained in puromycin-containing medium after strain application. To ensure that this concentration of puromycin was sufficient to inhibit protein synthesis, protein synthetic rate was estimated by measuring the rate of [3H]leucine incorporation into total protein. Cardiomyocytes plated at a density of 500 000 cells/cm² were maintained in control medium, or medium containing puromycin (10 μmol/L) or phorbol 12-myristate 13-acetate (PMA, 200 nmol/L, positive control) for 24 hours. [3H]Leucine (2 μCi/dish) was added during the last 6 hours of treatment. After washing with cold PBS, macromolecules were precipitated with ice-cold 10% trichloroacetic acid (TCA), and scraped from the dishes. The insoluble protein was then washed twice with TCA, and then solubilized (60°C, 20 minutes) in 200 μL of 0.2N NaOH. [3H]Leucine incorporation was determined by liquid scintillation counting. Protein concentration was determined by Bradford assay. Protein synthesis was described as DPM/μg of protein.

Adenoviral Constructs
Replication-defective adenoviruses (Adv) used in this study were propagated in HEK293 cells, and purified by double CsCl centrifugation as previously described.24 Adv were titrated by viral dilution in HEK293 cells grown in 96-well microtiter plates. PKCe activity was inhibited using an Adv encoding a dominant-negative mutant of rabbit PKCe (Adv-dPKCe), kindly provided by Dr Peipei Ping (University of California, Los Angeles).30 An Adv encoding cytoplasmic β-galactosidase (Adv-βgal) was used to control for non-specific effects of Adv infection. Infection efficiency was assessed in preliminary studies by immunohistochemical staining with monoclonal anti-β-gal antibody (Promega), as well as by immunohistochemical staining of the myc epitope tag (HA.11 polyclonal antibody PRB-101P, Covance Research Products) within the dnPKCe transgene. A multiplicity of viral infection (MOI) of ~50 was sufficient to infect ~90% of the cultured cells. Cardiomyocytes were infected 24 hours before uniaxial strain was applied.

F-AK-dependent signal transduction was inhibited by Adv-mediated overexpression of GFP-tagged FRNK, the autonomously expressed C-terminal region of FAK.21 Adv expressing GFP alone was used to control for nonspecific effects of Adv infection. Preliminary studies indicated that an MOI of ~10 was sufficient to infect ~90% of cells, based on GFP fluorescence. Cells were infected with either Adv-GFP or Adv-GFP-FRK 24 hours before strain, and then sarcomere length changes were monitored in randomly selected cells over time. Both Adv-dnPKCe and Adv-GFP-FRNK produced high levels of transgene overexpression, as previously analyzed in cardiomyocytes maintained in 2-D culture.31,32

Data Analysis
All values are mean±SEM. Data were compared using one-way and two-way ANOVA or the Students unpaired t test. Differences among means were considered significant at P<0.05. Data were analyzed using GraphPad statistical software.

Results
Membrane Strain
Silicone membranes were fabricated from a parylene template to generate a microtextured surface consisting of a combination of pegs and grooves (Figure 1A). To determine whether membrane strain was uniform, peg-to-peg distance was measured at representative positions across the membrane surface before and after 10% uniaxial stretch (Figure 1B). Actual strains at all five regions were similar after the applied strain (9.17±0.11%, n=3) or after gluing the membrane to the slide (9.19±0.07%, n=3) (Figure 1C). Slight transverse compression (~2.40±0.08%, n=3) was noted immediately after applying a 10% strain. This compression remained constant after gluing the membrane to the slide (~2.42±0.08%, n=3) (Figure 1D).

Growth of Cardiac Myocytes on Textured Surface
Cardiomyocytes grown on laminin-coated, flat surfaces were randomly oriented, as shown in the representative phase-contrast image depicted in Figure 2A. However, cardiomyocytes grown on microtextured surfaces were uniformly oriented along the long axis of the grooves (Figure 2B). In addition, sarcomeres (visualized by phalloidin staining) were randomly oriented in cardiomyocytes cultured on flat membranes (Figure 2C), as opposed to the aligned pattern on the microtextured surfaces (Figure 2D). Myofibrillar alignment was then quantified by image analysis. There was a high degree of alignment on textured surfaces, but not on flat surfaces before strain (flat, 6.0±2.6%, textured, 82±5.1%, P<0.001, n=3) and immediately after strain (flat, 9.0±3.5%, textured, 75±3.7%, P<0.001, n=3) (Figure 2E).

Regional Sarcomere Length Variation With Strain
We next examined the uniformity of sarcomere length within a myocyte at four different subcellular zones before and after strain (Figure 3). The four subcellular zones consisted of cells that (1) spanned the entire 100-μm length of the groove and
attached at both ends to pegs (Figure 3A), (2) attached to a peg at only one end (Figure 3B), (3) surrounded a peg (Figure 3C), or (4) were not attached to a peg at either end (Figure 3D). Cardiomyocytes were randomly selected and the sarcomere lengths were quantified and compared (Figure 3E). Sarcomere lengths accurately followed the 10% applied strain in every zone and there were no detectable inhomogeneities.

Recovery of Resting Sarcomere Length With Uniaxial Static Strain

The recovery of resting sarcomere length was used as an indicator of the cardiac remodeling process. Initial analysis of sarcomere length over a period of 48 hours showed that recovery of resting sarcomere length occurred by 6 hours (data not shown). Subsequently, we analyzed sarcomere length changes from 0 to 6 hours more extensively from more than 100 cardiomyocytes from 7 independent cultures at each time point. Resting sarcomere lengths on textured and flat surfaces were similar before strain was applied (1.94 ± 0.07 μm) (Figure 4). Sarcomere length increased to 2.06 ± 0.06 μm immediately after applying a nominal 10% static strain to the textured surface. Sarcomere length recovered by 4 hours on the textured surface (1.95 ± 0.17 μm). However, cardiomyocytes grown on flat surfaces never lengthened significantly after being strained 10% (1.95 ± 0.07 μm), and consequently were still at rest length at the 4-hour time point (1.98 ± 0.02 μm).

Effect of Translational Block on Sarcomere Length Recovery

To determine whether the restoration of resting sarcomere length required new protein synthesis, cells were treated with...
synthesis, as estimated by [3H]leucine incorporation into total protein studies were conducted to determine whether this puromycin (10 µmol/L) to inhibit mRNA translation. Preliminary studies were conducted to determine whether this puromycin concentration was sufficient to block protein synthesis, as estimated by [3H]leucine incorporation into total protein (Figure 5A). Using PMA, a known hypertrophic agonist, as a positive control, we found a 50% decrease in protein synthetic rate in cardiomyocytes treated with puromycin for 24 hours, whereas PMA showed a 10% increase compared with unstimulated, control cells.

Cardiomyocytes cultured on microtextured surfaces were then treated with puromycin for 30 minutes or 24 hours before strain (Figure 5B). In cells treated with puromycin for 30 minutes, we found an initial increase in sarcomere length (0.15 ± 0.07 µm; 10%, 2.06 ± 0.06 µm; *P<0.001). By 4 hours, the measured sarcomere length recovers on the textured (Combo) surface to 1.95 ± 0.17 µm. Myocytes cultured on a flat (Flat) membrane did not have a significant increase in sarcomere length when strained (n>100 cells from 7 independent cultures).

Cardiomyocytes cultured on microtextured surfaces were then infected with adenovirus to express dominant-negative PKCα or PKCβII to study the effects of PKC inhibition on sarcomere length recovery. Figure 4. Resting sarcomere length recovery. Sarcomere lengths were measured over time from randomly selected myocytes before and after strain. Within 5 minutes of strain of the textured (Combo) silicone membrane by a nominal 10%, the sarcomere length increased from a resting value of 1.94 ± 0.07 to 2.06 ± 0.06 µm (*P<0.001). By 4 hours, the measured sarcomere length recovers on the textured (Combo) surface to 1.95 ± 0.17 µm. Myocytes cultured on a flat (Flat) membrane did not have a significant increase in sarcomere length when strained (n>100 cells from 7 independent cultures).

Effect of PKC Inhibition on Recovery of Resting Cardiac Sarcomere Length

We next used the nonisoform-specific PKC inhibitors, chelerythrine chloride and staurosporine, to evaluate the influence of PKC on the recovery of resting sarcomere length. As seen in Figure 6A, both inhibitors prevented the recovery of resting sarcomere length after a 10% static stretch, suggesting a role for one or more PKC isoenzymes in the remodeling process.

PKCe is the major novel PKC isoenzyme expressed in cardiomyocytes. This isoenzyme has been implicated in both cytoskeletal remodeling, and the induction of specific aspects on cardiomyocyte hypertrophy. Therefore, we compared the ability of cardiomyocytes overexpressing a dominant-negative mutant of PKCe to undergo recovery of resting sarcomere length. Cells grown on microtextured surfaces were infected with Adv-dnPKCe (50 MOI, 24 hours before stretch). Adv-cβgal (50 MOI) was used to control for non-specific effects of Adv infection. Sarcomere length acutely increased in cells infected with either Adv-cβgal or Adv-dnPKCe (Figure 6B). However, only cells infected with Adv-cβgal were able to recover to their resting sarcomere lengths within the 4-hour recovery period (*P<0.01). These results indicate that PKCe is a critical component of the signaling pathways responsible for resting sarcomere length recovery during cardiomyocyte remodeling.

FAK-Mediated Regulation of Resting Sarcomere Length Recovery Assessed With FRNK

We next examined whether GFP-FRNK overexpression affected the recovery of resting sarcomere length in response to static strain. The control GFP transgene (Figure 7A) was diffusely localized within the cardiomyocyte. However, by capturing optical sections (0.1 µm) throughout the thickness of the cell and at the cell-substratum interface, we verified...
that GFP-FRNK localized to costameric structures and focal adhesions, as previously described (Figure 7B). Exchange of endogenous FAK with its truncated form, FRNK, subsequently prevented the recovery of resting sarcomere length following 10% strain (Figure 7C). The failure to recover resting sarcomere length in Adv-GFP-FRNK–infected myocytes was not due to nonspecific effects of adenoviral infection, as length recovery proceeded normally in cells infected with Adv-GFP.

**Discussion**

Our first novel finding indicates that the neonatal rat cardiomyocyte can add approximately one sarcomere per hour and effectively compensate for an increase in resting sarcomere length after an applied strain of 10%. We calculate this rate by knowing the following facts: a cardiomyocyte spans the length after an applied strain of 10%. We calculate this rate effectively compensate for an increase in resting sarcomere length. A PKCε-dependent signaling pathway appears critical in linking external mechanical stimulation to the internal environment. Our third finding is that the focal adhesion complex, through FAK, is also necessary for the cardiac remodeling process.

It has been difficult to design experiments to determine the cellular basis of length regulation in vivo. Cardiac atrophy and subsequent hypertrophy in vivo was previously demonstrated by severing and then reattaching the chordae tendinea of a single right ventricular cat papillary muscle to alter the load on the myocytes. Cell shape changed creating a longer cell but sarcomere length was not measured. Cardiomyocyte dimensions also change, cross-sectionally and longitudinally, in response to increases in pressure or volume, respectively. Left ventricular myocytes isolated from patients with ischemic cardiomyopathy were shown to have longer and thinner cellular dimensions than cells from control tissue, but had normal resting sarcomere lengths, again suggesting that rest length is a tightly controlled process.

Although cyclic stretching of cardiomyocytes on a flat surface results in changes in overall cell size, this in vitro model system provides only limited information about sarcomere length changes, and thus limited insight regarding the regulatory mechanisms involved in load-induced structural remodeling. Our findings suggest that the average sarcomere length of cardiomyocytes grown on flat, laminin-coated surfaces does not change when strained. Because these cells were randomly oriented, only a small fraction of the cardiomyocytes was precisely oriented in the direction of the applied vector. In addition, earlier work by our group using similarly fabricated, textured surfaces demonstrated that focal adhesion complexes, such as paxillin and vinculin, were expressed at levels similar to those observed in the intact, neonatal heart. In particular, we suspect that the vertical topography of the 3-D microtextured surface provides a better geometry for anchorage, and thus an increase in the number of focal adhesion complexes necessary for cell attachment. However, anchorage to the vertical pegs was not required for the transmission of length changes from the membrane to the sarcomeres, as sarcomeric extension was similar in cells lying in the grooves that were not attached to the pegs. We suspect that the vertical costameres on the sidewalls of the grooves are also important topographical features for efficient mechanontransduction. Thus, our microtextured surface consisting of both pegs and grooves has unique advantages for mechanoochemical signaling studies, especially with respect to the role of the focal adhesion complex.

In addition, we previously showed that adenovirally mediated overexpression of GFP-FRNK in nonaligned cultures of neonatal rat ventricular myocytes caused the displacement of endogenous FAK from focal adhesions within 24 hours. FRNK overexpression also markedly inhibited cell spreading and sarcomeric assembly in response to endothelin-1 added to the culture medium, and prevented the PKCε-dependent autophosphorylation of FAK. Furthermore, we recently demonstrated that focal adhesion proteins accumulate near each peg in aligned myocyte cultures, suggesting that focal adhesion complexes may be important sites for the both structural remodeling and mechanoochemical signaling.
A cell contributes to mRNA accumulation near the site of the focal adhesion complex. This evidence supports the hypothesis that control of sarcomeric protein addition in cardiac muscle, as similarly shown in skeletal muscle models with mechanotransduction signaling cascades.

FAK is closely associated with a number of other key cytoskeletal proteins, such as paxillin, and critical kinases such as Rho kinase and Src. More work needs to be done to determine the effect FAK has on these other supporting proteins during cardiac remodeling. However, with the evidence we have provided, we can conclude that FAK plays a critical role in the cellular response to mechanical stimulation. It is likely that a mechanical signal from the extracellular environment is transduced through the focal adhesion complex during mechanical deformation triggering a signaling cascade that could travel to Z discs for mechanical coordination of cytoskeletal remodeling. Intracellular cytoskeleton organization has been shown to depend on mechanical stimulation.

In conclusion, our study investigated the remodeling of resting sarcomere length in cardiac myocytes as seen in in vivo situations of volume overload. An important and novel finding in our study is the addition of about one sarcomere per hour when myocytes are strained 10% to compensate for the increase in resting sarcomere length. These additional sarcomeres are sufficient to return the cell to an optimal sarcomere rest length. In addition, our study suggests that the regulation of the resting sarcomere length recovery process is mediated by PKC and FAK.

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References

Our work also contributes to the growing body of evidence implicating PKC isoenzymes as mediators of cardiac remodeling. The present study does not indicate a direct association between PKC and FAK, nor does it indicate whether the two signaling kinases reside in the same, or parallel stretch-activated signaling pathways. However, previous studies from our group have demonstrated that PKC lies upstream of FAK in a signaling pathway activated in response to endothelin-1 receptor stimulation in neonatal rat cardiomyocytes. Thus, it is conceivable that PKC and FAK reside in a similar pathway activated in response to mechanical load. FAK, in turn, activates a number of crucial downstream signaling cascades that regulate cell spreading, focal adhesion formation, cytoskeletal reorganization, and gene expression changes. Inhibition of PKC- and FAK-dependent signaling may have interfered with the earliest steps in mechanochemical signaling, and therefore prevented resting sarcomere length recovery.

It is also conceivable that PKC and FAK are involved more directly in structural remodeling. Focal adhesion complexes are found both at the periphery and along costameric grooves further supports the differential regulation of gene expression seen in longitudinal and transverse aligned cells associated with mechanotransduction signaling cascades.


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