Biochemical and Biophysical Research Communications 372 (2008) 216–220

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Pulsatile equibiaxial stretch inhibits thrombin-induced RhoA and NF-κB activation

Jason H. Haga [a,*], Roland Kaunas [a], Julie Radeff-Huang [b], Jessica M. Weems [b], Kristine D. Estrada [b], Shu Chien [a,c], Joan Heller Brown [b], Tammy M. Seasholtz [b]

[a] Department of Bioengineering, University of California, San Diego, 9500 Gilman Drive, La Jolla, CA 92093-00435, USA
[b] Department of Pharmacology, University of California, San Diego, 9500 Gilman Drive, La Jolla, CA 92093-00435, USA
[c] Department of Medicine, University of California, San Diego, 9500 Gilman Drive, La Jolla, CA 92093-00435, USA

ARTICLE INFO

Article history:
Received 1 May 2008
Available online 12 May 2008

Keywords:
Mechanical stretch
RhoA
Thrombin
Vascular smooth muscle
NF-κB

ABSTRACT

This study investigated interactions between the effects of mechanical stretch and thrombin on RhoA activation in rat aortic smooth muscle cells (RASMC). Equibiaxial, pulsatile stretch, or thrombin produced a significant increase in RhoA activation. Surprisingly, in combination, 30 min of stretch inhibited the ability of thrombin to activate RhoA. NO donors and 8-bromo-cGMP significantly inhibited thrombin-induced RhoA activation. Interestingly, the nitric oxide synthase (NOS) inhibitor L-NAME increased basal RhoA activity, suggesting that NOS activity exerts a tonic inhibition on RhoA. Stretching RASMC increases nitrite production, consistent with the idea that NO contributes to the inhibitory effects of stretch. Thrombin stimulates MAP kinase and NF-κB pathways through Rho and these responses were blocked by 8-bromo-cGMP or stretch and restored by L-NAME. These data suggest that stretch, acting through NO and cGMP, can prevent the ability of thrombin to stimulate Rho signaling pathways that contribute to pathophysiological proliferative and inflammatory responses.

Published by Elsevier Inc.

The role of Rho GTPases in regulation of cell morphology [1], vascular smooth muscle contraction [2], and cell migration [3] is well recognized. Equally important but less well studied is the involvement of RhoA in cell proliferation [3,4] and inflammatory responses [5,6]. Enhanced proliferative and migratory capacity of vascular smooth muscle cells (VSMC), coupled with an increased predisposition toward inflammation, characterize many blood vessel diseases, and growing evidence suggests that alterations in RhoA expression, activity, and/or signaling play a role in a subset of vascular disorders [7].

NOS mediated NO production and activation of the guanylyl cyclase-cGMP pathway have been widely demonstrated to regulate vascular smooth muscle contraction, proliferation, and migration [8]. In contrast to the stimulatory effects of RhoA, the effect of activating the NO pathway is to inhibit each of these responses. Increasing Rho activity has been shown to reduce eNOS mRNA stability [9], and it has been suggested that the effects of statins to upregulate both eNOS and iNOS result from Rho inhibition [9,10].

The G protein-coupled receptors (GPCRs) that signal through the $G_{12/13}$ family of heterotrimeric G proteins have well-documented effects on RhoA activation. Prominent among these are the receptors for thrombin, lysophospholipids, and thromboxane A_2 [11]. Our previous reports demonstrated that acute stimulation of RASMC with thrombin produced significant increases in membrane-associated RhoA, as well as RhoA-[^{35}S]GTPγS binding and RhoA activation [3,12]. In addition to agonist stimulation of Rho pathways, mechanical forces also regulate Rho [13–15]. Numaguchi and colleagues have shown that cyclic (i.e., pulsatile), mechanical stretch with a magnitude of 20% activates Rho in RASMC [14] and our laboratory demonstrated that static stretch-induced Rho activation in neonatal rat ventricular myocytes [15]. While the mechanisms for activation of RhoA in response to GPCR stimulation or mechanical forces have been investigated [13,16,17], the interaction of mechanical and hormonal stimuli in regulating RhoA activation has not been addressed.

VSMC *in vivo* are exposed to constant pulsatile stretch as a result of the cardiac cycle and are simultaneously regulated by circulating and locally derived neurohumoral factors. In this study, we examined the interactions between pulsatile, equibiaxial stretch, and thrombin, an agonist that elicits RhoA activation and Rho-dependent responses in RASMC [3,4]. The data provide evidence that stretch can inhibit RhoA activation by acting through NOS/cGMP, thereby attenuating agonist-induced Rho-dependent signaling pathways involved in cell proliferation and gene expression.

* Corresponding author. Fax: +1 858 822 1160.
E-mail address: jhaga@bioeng.ucsd.edu (J.H. Haga).

0006-291X/$ - see front matter Published by Elsevier Inc.
doi:10.1016/j.bbrc.2008.05.013

Methods

Cell culture. RASMC were isolated and cultured from the thoracic aorta of adult, male Sprague–Dawley rats as previously described [3]. The cells were maintained in high glucose DMEM supplemented with 1% L-glutamine, 1% penicillin/streptomycin, and 20% BSA in a 5% CO_2 incubator. Cells between passages three and eight were passed 4–7 days prior to assay, grown to 50–75% confluence, and serum-starved overnight.

Pulsatile equibiaxial stretch. RASMC were stretched using a device previously described [18,19]. Briefly, silicone membranes were attached to polycarbonate chambers with O-rings. The chambers were sterilized with UV light and coated with 1 μg/cm^2 fibronectin (Sigma, St. Louis, MO) overnight. RASMC were seeded onto the membranes at 3125 cells/cm^2, grown until 50–60% confluent, and serum-starved overnight prior to stretching. All controls were time-matched, sham controls. The stretching device was set to produce a 10% linear stretch at a frequency of 1 Hz. Shear stresses due to the movement of fluid in the stretch chamber are estimated to be no more than 0.2 dynes/cm^2 and do not affect the signaling pathways investigated in this study [20]. The entire device was placed inside an incubator to maintain 100% humidity, 5% CO_2, and 37 °C.

Immunoblot Analysis. RASMC were lysed with a buffer containing 10 mM Tris–HCl, 150 mM NaCl, 2.5 mM EDTA, 10% glycerol, 1% Igepal/NP-40, 50 mM NaF, 20 mM Na pyrophosphate, 10 μg/mL aprotinin, 10 μg/mL leupeptin, 2 mM Na_3VO_4, and 1 mM PMSF. Lysates were clarified by centrifuging and protein concentration was obtained by the Bradford protein assay. Following SDS–PAGE and protein transfer, the PVDF membranes were blocked with 5% milk or 3% BSA in TBS/Tween 20, and then exposed to either p-ERK antibody (Cell Signaling Technology, Beverly, MA) or p-IκBα antibody (Santa Cruz Biotechnology, Santa Cruz, CA) at 4 °C overnight. Following incubation with the appropriate secondary antibody, proteins were detected using enhanced chemiluminescence and analyzed by gel documentation imaging.

RhoA activation. RASMC were stretched for various times as indicated and/or exposed to vehicle or thrombin for 3 min prior to washing with PBS and lysis as previously described [12]. Lysates were subjected to affinity precipitation assay using the GST-fusion protein of the Rho binding domain of rhotekin (a generous gift from Dr. Martin Schwartz, The University of Virginia, Charlottesville, VA). The samples were then immunoblotted for active RhoA as previously described [12].

Nitrite Assay. One day before the experiment, RASMC were serum-starved overnight with phenol red-free DMEM supplemented with 1% L-glutamine, 1% penicillin/streptomycin, 0.1% BSA, and 1.5 mM L-arginine. On the following day, cells were exposed to 10% pulsatile, equibiaxial stretch or thrombin for the times indicated. Nitrite in the media was measured using a Greiss reagent system (Promega) as previously described [21], and the absorbance of the samples was determined at 520 nm.

Statistical analysis. Data were analyzed by one-way ANOVA or Student's *t*-test where appropriate. For ANOVA, post-analysis was performed with the Tukey test using $p < 0.05$.

Results

Pulsatile, equibiaxial stretch inhibits thrombin-induced RhoA activation

To examine the effect of mechanical stretch on RhoA activation, RASMC were harvested after 5 min to 4 h of stretch and subjected to rhotekin RBD affinity precipitation assay [12]. Five or 30 min of stretch produced modest but significant increases in RhoA activation, which diminished by 1 h of stretch and completely returned to baseline levels by 4 h (Fig. 1A). Thrombin alone produced a robust fivefold increase in RhoA activation (Fig. 1B), consistent with previous observations [3,12]. Interestingly, when cells had been pre-exposed to stretch for 30 min, the addition of thrombin did not further activate RhoA, but instead stretch fully prevented thrombin-induced activation of RhoA (Fig. 1B).

The NO-cGMP pathway inhibits thrombin-induced RhoA activation

Inhibition of thrombin-induced RhoA activation could also be elicited by stimulation of the NO-cGMP pathway. Pretreatment of RASMC with either a NO donor (PAPA-NONOate 250 μM) or 8-bromo-cGMP (500 μM) for 4 h led to a significant reduction in thrombin-induced RhoA activation (Fig. 2A). Conversely, inhibition of NOS by L-NAME treatment (10 mM) for 24 h led to a significant increase in basal levels of active RhoA (Fig. 2B).

Since mechanical forces such as shear stress have been reported to increases NO release from endothelial cells [22], we postulated that pulsatile, equibiaxial stretch might increase NO release from vascular smooth muscle cells. After 30 min of stretch, RASMC produced a significant increase in nitrite production (Fig. 2C) indicative of NOS-generated NO production. Thus, NO could attenuate the rise in RhoA activation in response to stretch and could contribute to the inhibition of thrombin-induced RhoA activation. Nitrite production under basal state and induced by stretch was L-NAME-sensitive (data not shown), indicating that NOS is responsible for the observed nitrite accumulation.

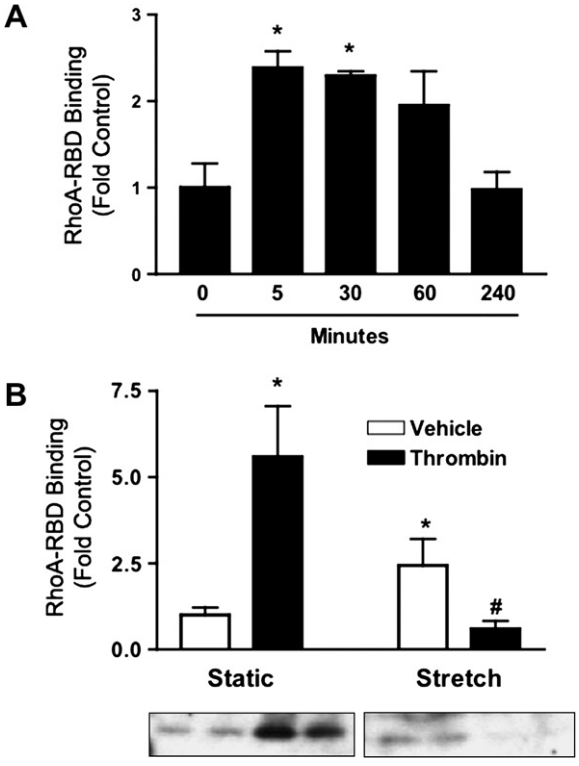

Fig. 1. Mechanical stretch stimulates RhoA activation, but inhibits thrombin-induced RhoA activation. (A) The increase in active RhoA following mechanical stretch of cells for 5–240 min. (B, left panel) The marked increase in RhoA activation by thrombin (12 nM for 3 min). (B, right panel) 30 min of pre-stretch reversed the effect of thrombin on RhoA activation from stimulation to inhibition. Data represent means and standard errors from three experiments. $^{*}p < 0.01$ vs control. $^{\#}p < 0.01$ vs static thrombin.

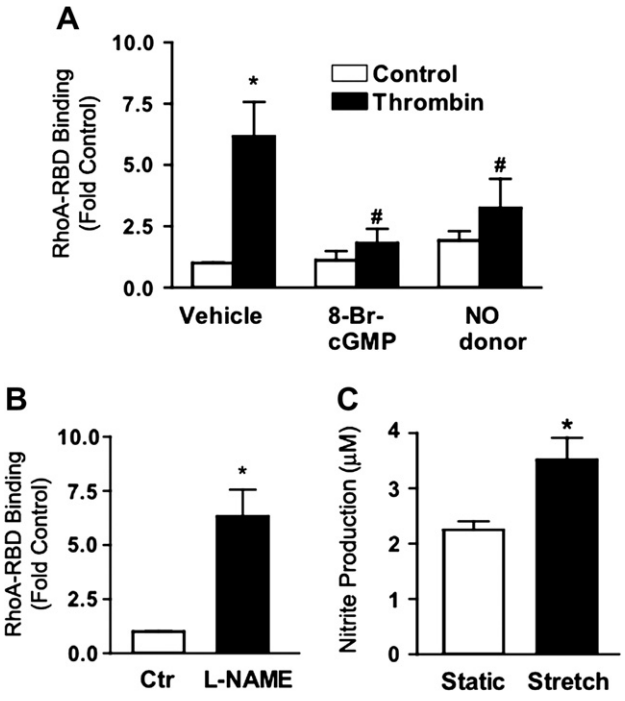

Fig. 2. The NO-cGMP pathway regulates RhoA activation in vascular smooth muscle cells. (A) Cells were treated with either 8-bromo-cGMP (500 mM) or PAPA-NONOate (250 mM) for 4 h prior to stimulation with thrombin for 5 min and measurement of active RhoA. Both 8-bromo-cGMP and PAPA-NONOate attenuated the thrombin-induced RhoA activation. (B) Cells were treated overnight with L-NAME (10 mM) and active RhoA was assayed. L-NAME caused a marked increase in active RhoA. Data represent means and errors from three experiments. $^{*}p < 0.01$ vs control. $^{\#}p < 0.01$ vs thrombin pretreated with vehicle. (C) The Greiss reagent was used to detect changes in nitrite production by RASMC following 30 min of pulsatile, equibiaxial stretch. Data represent means and standard errors from three experiments. $^{*}p < 0.01$ vs static.

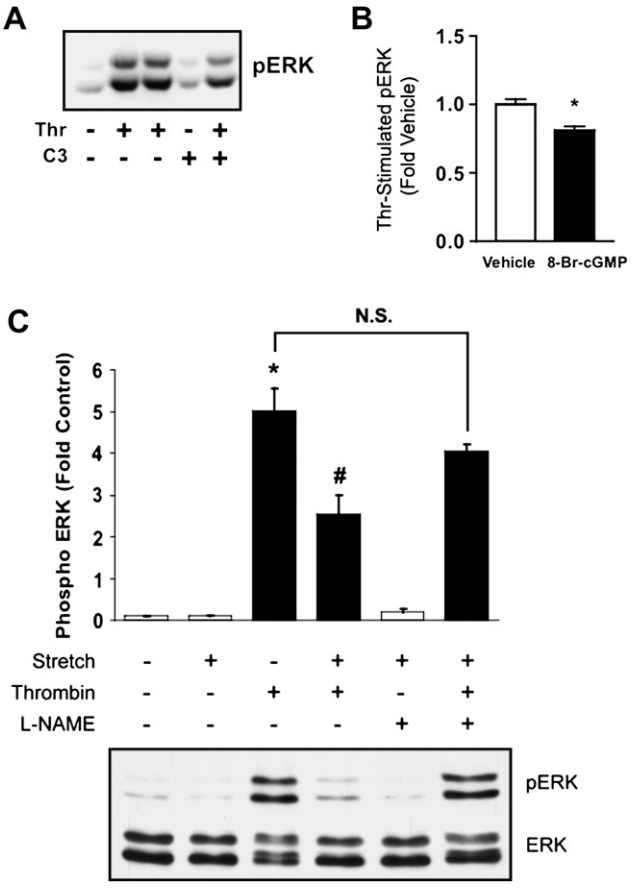

Fig. 3. C3, 8-bromo-cGMP and stretch inhibit, while L-NAME restores thrombin-induced ERK phosphorylation. RASMC were treated with either C3 (5 ng/ml) overnight (A) or 8-bromo-cGMP (500 mM) for 4 h (B) prior to stimulation with thrombin (12 nM) for 5 min and assayed for ERK phosphorylation. The thrombin-induced ERK phosphorylation was attenuated by C3 and 8-bromo-cGMP. (A) Representative of two experiments with a total of 3–4 replicates. (B) The means and standard errors from two experiments performed in duplicate. $^{*}p < 0.001$. (C) RASMC were treated with L-NAME for 4 h followed by stretch for 30 min with addition of vehicle or thrombin for the last 5 min. Data represent mean and standard errors from four experiments. $^{*}p < 0.05$ vs vehicle alone. $^{\#}p < 0.05$ vs static thrombin. The thrombin-induced ERK phosphorylation was attenuated by stretch, and this attenuation was blocked by L-NAME. There was no significant difference (NS) in the phosphorylation of ERK between thrombin alone and thrombin with stretch in the presence of L-NAME.

Pulsatile, equibiaxial stretch inhibits, while L-NAME restores, thrombin-induced, Rho-dependent ERK phosphorylation

To determine whether stretch also effected Rho-dependent proliferative signaling, we examined ERK activation [23]. Treatment of RASMC with thrombin for 5 min produced a significant increase in ERK activation (Fig. 3A). Overnight treatment of cells with the Rho inhibitor C3 exoenzyme, produced a partial decrease in thrombin-stimulated p-ERK, suggesting involvement of Rho (Fig. 3A). Pretreatment of RASMC with 8-bromo-cGMP also produced a partial decrease in thrombin-stimulated ERK phosphorylation (Fig. 3B). Thrombin-induced ERK phosphorylation was attenuated by 30 min of stretch, and pretreatment of cells with L-NAME prevented this inhibition (Fig. 3C).

Pulsatile, equibiaxial stretch inhibits, while L-NAME restores, thrombin-induced, Rho-dependent IκBα phosphorylation

NF-κB is a major player in regulating gene expression in inflammation, and its activation via IKK-dependent phosphorylation of IκB-α utilizes Rho-dependent pathways [24]. We asked whether stretch would effect activation of NF-κB-regulated signaling pathways (assayed by IκB-α phosphorylation) through Rho inhibition. Thrombin produced a significant increase in IκB-α phosphorylation at 5 min (Fig. 4A), which returns to baseline by 15 min (data not shown). Treatment of cells with C3 exoenzyme or with 8-bromo-cGMP caused a complete inhibition of thrombin-induced IκB-α phosphorylation (Fig. 4A). These results provide evidence that the NO-cGMP pathway negatively regulates Rho-dependent NF-κB activation. When RASMC were exposed to 30 min of stretch and treated with thrombin for 5 min, IκB-α phosphorylation was significantly reduced (Fig. 4B). Treatment with L-NAME to inhibit NO production abolished the effect of stretch on thrombin-induced IκB-α phosphorylation, as in the case with thrombin-induced ERK phosphorylation.

Discussion

This study examined the combined effect of mechanical and hormonal stimuli on growth promoting and inflammatory signal transduction in VSMC. Our results show that both the GPCR agonist thrombin and pulsatile, equibiaxial stretch produce significant increases in RhoA activation, although the response to stretch was less pronounced than that of thrombin. When administered in combination, however, stretch not only blocked the RhoA activation induced by thrombin, but actually reversed the effect to one of inhibition. This inhibitory effect of stretch was mediated, in part, through an NO-cGMP mechanism. Our data also indicate that VSMC possess a basal level of NOS activity that provides a tonic

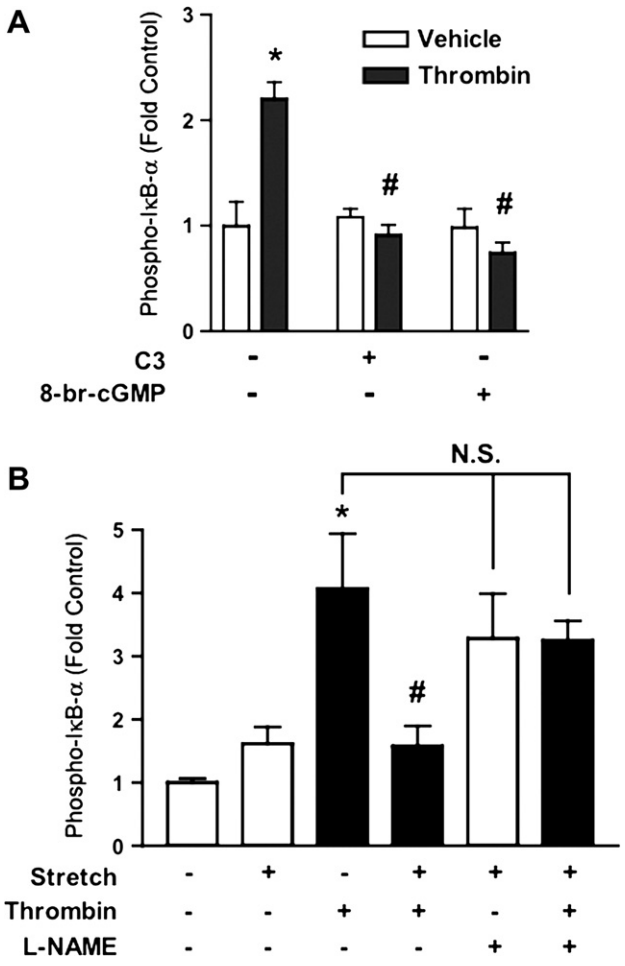

Fig. 4. C3, 8-bromo-cGMP, and stretch inhibit, while L-NAME restores thrombin-induced IκB-α phosphorylation. (A) RASMC were treated with either C3 (5 ng/ml) overnight or 8-bromo-cGMP (500 mM) for 4 h prior to assay for IκB-α phosphorylation using immunoblotting. Data represent means and standard errors from four experiments. The thrombin-induced IκB-α phosphorylation was blocked by C3 and 8-bromo-cGMP. $^{*}p < 0.01$ vs vehicle alone. $^{\#}p < 0.01$ vs static thrombin. (B) RASMC were treated with L-NAME for 4 h followed by stretch for 30 min with addition of vehicle or thrombin for the last 5 min. Data represent means and standard errors from four experiments. The thrombin-induced IκB-α phosphorylation was attenuated by stretch, and this attenuation was inhibited by L-NAME. $^{*}p < 0.01$ vs vehicle alone. $^{\#}p < 0.01$ vs static thrombin. There was no significant difference (NS) between static thrombin and thrombin with stretch in the presence of L-NAME.

inhibition of RhoA activity and this is further augmented by stretch.

Mechanical stretch not only inhibited the thrombin-induced RhoA activation, but it also reduced thrombin-induced, Rho-dependent responses such as ERK and NF-κB activation. Similar evidence has been reported in mesangial cells where NO inhibits stretched-induced MAPK activation via RhoA inactivation [25]. Studies also suggest that a number of signaling cascades resulting from Rho activation contribute to VSMC proliferation and inflammation. For proliferative responses, these Rho-dependent signals include regulating translocation of ERK from the cytosol to the nucleus in response to static stretch [15] or GPCR activation [26]. In some systems, ERK phosphorylation has also been shown to be dependent upon the Rho–Rho kinase-LIM kinase pathway [23]. Evidence increasingly suggests that Rho signaling can also regulate inflammation through activation of NF-κB signaling. Activations of NF-κB by TNF-α, fMet-Leu-Phe, and thrombin have all been shown to require Rho [5,6,24].

Other studies have investigated the effect of both mechanical and biochemical stimuli on vascular endothelial cell responses [27–29]. The current study extends these investigations to VSMC, which have not been studied in this context. Importantly, inhibition of thrombin-stimulated ERK phosphorylation and IκB-α phosphorylation by stretch was prevented by treatment with the NOS inhibitor L-NAME, providing support for a role of NOS/NO in stretch-induced inhibition of Rho and Rho-dependent VSMC responses.

The findings presented here have important implications regarding the interaction of mechanical forces and hormonal factors on Rho signaling in vascular diseases. Kaibuchi et al. have shown that blood vessel contraction in the absence of increasing intracellular Ca^{2+} is mediated by Rho [2], and involves Rho kinase phosphorylation of and inhibition of myosin phosphatase [30]. Contraction, proliferation and inflammation are thus all Rho-dependent cellular responses that are dysregulated in vascular diseases. Although mechanical stretch has been reported to have both proliferative and anti-proliferative effects on VSMC (see [31], for review), the observation that stretch can inhibit early Rho-dependent signaling events involved in proliferative and inflammatory signaling suggest that it plays a critical role in providing protection against the development of vascular dysfunction.

It is tempting to hypothesize that during states of reduced NO generation or increased Rho activity, stretch would have a greatly reduced ability to inhibit Rho-dependent signaling. Enhanced vascular RhoA expression, RhoA activity, Rho kinase activity, and/or Rho-dependent DNA synthesis have been reported in vessels and smooth muscle cells from spontaneously hypertensive, NO-deficient, and mineralocorticoid hypertensive rats [12,32–34]. Similar increases in vascular Rho activation have also been described following stenting of human mammary vessels [35]. In these pathophysiological conditions, stretch-generated NO might be insufficient to keep RhoA signaling in check. Consistent with this theory, dominant negative Rho kinase or statins have been shown to have inhibitory effects on neointimal formation following vascular injury [36,37] and atheroma formation [38].

In summary, the present study provides evidence for cross-talk between signaling pathways activated by mechanical and hormonal stimuli. Any deviation of the physiologic environment from the normal state, such as that observed in endothelial denudation, decreased NO production, or upregulation of Rho, could lead to increased proliferation and inflammation, thus contributing to the progression of vascular disease. The inhibition of thrombin-induced RhoA activation by stretch suggests that mechanical forces provide an additional level of regulation of cell proliferation and inflammatory signaling.

Acknowledgments

J.H.H. is a recipient of a NIH Ruth L. Kirschstein Individual NRSA (HL71390). T.M.S. is a recipient of an AHA National Scientist Development Grant. This study was supported in part by NIH Grants GM36927 (J.H.B.), HL64382 (S.C.), HL080518 (S.C.), and HL43026 (S.C.).

References

[1] A.J. Ridley, A. Hall, The small GTP-binding protein rho regulates the assembly of focal adhesions and actin stress fibers in response to growth factors, Cell 70 (1992) 389–399.
[2] K. Hirata, A. Kikuchi, T. Sasaki, S. Kuroda, K. Kaibuchi, Y. Matsuura, H. Seki, K. Saida, Y. Takai, Involvement of rho p21 in the GTP-enhanced calcium ion sensitivity of smooth muscle contraction, J. Biol. Chem. 267 (1992) 8719–8722.
[3] T.M. Seasholtz, M. Majumdar, D.D. Kaplan, J.H. Brown, Rho and Rho kinase mediate thrombin-stimulated vascular smooth muscle cell DNA synthesis and migration, Circ. Res. 84 (1999) 1186–1193.
[4] M.F. Olson, H.F. Paterson, C.J. Marshall, Signals from Ras and Rho GTPases interact to regulate expression of p21Waf1/Cip1, Nature 394 (1998) 295–299.
[5] S. Hippenstiel, B. Schmeck, J. Seybold, M. Krull, C. Eichel-Streiber, N. Suttorp, Reduction of tumor necrosis factor-alpha (TNF-alpha) related nuclear factor-

kappaB (NF-kappaB) translocation but not inhibitor kappa-B (Ikappa-B)-degradation by Rho protein inhibition in human endothelial cells, Biochem. Pharmacol. 64 (2002) 971–977.
[6] L.Y. Chen, B.L. Zuraw, R.D. Ye, Z.K. Pan, A Rho exchange factor mediates fMet-Leu-Phe-induced NF-kappaB activation in human peripheral blood monocytes, J. Biol. Chem. 279 (2004) 7208–7212.
[7] T.M. Seasholtz, J.H. Brown, RHO SIGNALING in vascular diseases, Mol. Interv. 4 (2004) 348–357.
[8] R. Feil, S. Feil, F. Hofmann, A heretical view on the role of NO and cGMP in vascular proliferative diseases, Trends Mol. Med. 11 (2005) 71–75.
[9] U. Laufs, J.K. Liao, Post-transcriptional regulation of endothelial nitric oxide synthase mRNA stability by Rho GTPase, J. Biol. Chem. 273 (1998) 24266–24271.
[10] M. Hausding, A. Witteck, F. Rodriguez-Pascual, C. von Eichel-Streiber, U. Forstermann, H. Kleinert, Inhibition of small G proteins of the rho family by statins or clostridium difficile toxin B enhances cytokine-mediated induction of NO synthase II, Br. J. Pharmacol. 131 (2000) 553–561.
[11] N.A. Riobo, D.R. Manning, Receptors coupled to heterotrimeric G proteins of the G12 family, Trends Pharmacol. Sci. 26 (2005) 146–154.
[12] T.M. Seasholtz, T. Zhang, M.R. Morissette, A.L. Howes, A.H. Yang, J.H. Brown, Increased expression and activity of RhoA are associated with increased DNA synthesis and reduced p27(Kip1) expression in the vasculature of hypertensive rats, Circ. Res. 89 (2001) 488–495.
[13] E. Tzima, M.A. del Pozo, S.J. Shattil, S. Chien, M.A. Schwartz, Activation of integrins in endothelial cells by fluid shear stress mediates Rho-dependent cytoskeletal alignment, EMBO J. 20 (2001) 4639–4647.
[14] K. Numaguchi, S. Eguchi, T. Yamakawa, E.D. Motley, T. Inagami, Mechanotransduction of rat aortic vascular smooth muscle cells requires RhoA and intact actin filaments, Circ. Res. 85 (1999) 5–11.
[15] S. Kawamura, S. Miyamoto, J.H. Brown, Initiation and transduction of stretch-induced RhoA and Rac1 activation through caveolae: cytoskeletal regulation of ERK translocation, J. Biol. Chem. 278 (2003) 31111–31117.
[16] A.J. Putnam, J.J. Cunningham, B.B. Pillemer, D.J. Mooney, External mechanical strain regulates membrane targeting of Rho GTPases by controlling microtubule assembly, Am. J. Physiol. Cell Physiol. 284 (2003) C627–C639.
[17] M.J. Hart, X. Jiang, T. Kozasa, W. Roscoe, W.D. Singer, A.G. Gilman, P.C. Sternweis, G. Bollag, Direct stimulation of the guanine nucleotide exchange activity of p115 RhoGEF by Galpha13, Science 280 (1998) 2112–2114.
[18] M. Sotoudeh, S. Jalali, S. Usami, J.Y. Shyy, S. Chien, A strain device imposing dynamic and uniform equi-biaxial strain to cultured cells, Ann. Biomed. Eng. 26 (1998) 181–189.
[19] R. Kaunas, P. Nguyen, S. Usami, S. Chien, Cooperative effects of Rho and mechanical stretch on stress fiber organization, Proc. Natl. Acad. Sci. USA 102 (2005) 15895–15900.
[20] R. Kaunas, S. Usami, S. Chien, Regulation of stretch-induced JNK activation by stress fiber orientation, Cell Signal. 18 (2006) 1924–1931.
[21] A.B. Gustafsson, L.L. Brunton, Beta-adrenergic stimulation of rat cardiac fibroblasts enhances induction of nitric-oxide synthase by interleukin-1beta via message stabilization, Mol. Pharmacol. 58 (2000) 1470–1478.
[22] G.M. Rubanyi, J.C. Romero, P.M. Vanhoutte, Flow-induced release of endothelium-derived relaxing factor, Am. J. Physiol. 250 (1986) H1145–H1149.
[23] C.F. Welsh, K. Roovers, J. Villanueva, Y. Liu, M.A. Schwartz, R.K. Assoian, Timing of cyclin D1 expression within G1 phase is controlled by Rho, Nat. Cell Biol. 3 (2001) 950–957.
[24] K.N. Anwar, F. Fazal, A.B. Malik, A. Rahman, RhoA/Rho-associated kinase pathway selectively regulates thrombin-induced intercellular adhesion molecule-1 expression in endothelial cells via activation of I kappa B kinase beta and phosphorylation of RelA/p65, J. Immunol. 173 (2004) 6965–6972.
[25] J.C. Krepinsky, A.J. Ingram, D. Tang, D. Wu, L. Liu, J.W. Scholey, Nitric oxide inhibits stretch-induced MAPK activation in mesangial cells through RhoA inactivation, J. Am. Soc. Nephrol. 14 (2003) 2790–2800.
[26] Y. Liu, Y.J. Suzuki, R.M. Day, B.L. Fanburg, Rho kinase-induced nuclear translocation of ERK1/ERK2 in smooth muscle cell mitogenesis caused by serotonin, Circ. Res. 95 (2004) 579–586.
[27] N. Lerner-Marmarosh, M. Yoshizumi, W. Che, J. Surapisitchat, H. Kawakatsu, M. Akaike, B. Ding, Q. Huang, C. Yan, B.C. Berk, J. Abe, Inhibition of tumor necrosis factor-[alpha]-induced SHP-2 phosphatase activity by shear stress: a mechanism to reduce endothelial inflammation, Arterioscler. Thromb. Vasc. Biol. 23 (2003) 1775–1781.
[28] H. Yamawaki, S. Pan, R.T. Lee, B.C. Berk, Fluid shear stress inhibits vascular inflammation by decreasing thioredoxin-interacting protein in endothelial cells, J. Clin. Invest. 115 (2005) 733–738.
[29] Y. Wang, J. Chang, Y.C. Li, Y.S. Li, J.Y. Shyy, S. Chien, Shear stress and VEGF activate IKK via the Flk-1/Cbl/Akt signaling pathway, Am. J. Physiol. Heart Circ. Physiol. 286 (2004) H685–H692.
[30] K. Kimura, M. Ito, M. Amano, K. Chihara, Y. Fukata, M. Nakafuku, B. Yamamori, J. Feng, T. Nakano, K. Okawa, A. Iwamatsu, K. Kaibuchi, Regulation of myosin phosphatase by Rho and Rho-associated kinase (Rho-kinase), Science 273 (1996) 245–248.
[31] J.H. Haga, Y.S. Li, S. Chien, Molecular basis of the effects of mechanical stretch on vascular smooth muscle cells, J. Biomech. 40 (2007) 947–960.
[32] S. Chrissobolis, C.G. Sobey, Evidence that Rho-kinase activity contributes to cerebral vascular tone in vivo and is enhanced during chronic hypertension: comparison with protein kinase C, Circ. Res. 88 (2001) 774–779.
[33] D.S. Weber, R.C. Webb, Enhanced relaxation to the rho-kinase inhibitor Y-27632 in mesenteric arteries from mineralocorticoid hypertensive rats, Pharmacology 63 (2001) 129–133.
[34] T. Seko, M. Ito, Y. Kureishi, R. Okamoto, N. Moriki, K. Onishi, N. Isaka, D.J. Hartshorne, T. Nakano, Activation of RhoA and inhibition of myosin phosphatase as important components in hypertension in vascular smooth muscle, Circ. Res. 92 (2003) 411–418.
[35] P. Guerin, V. Sauzeau, M. Rolli-Derkinderen, O. Al Habbash, E. Scalbert, D. Crochet, P. Pacaud, G. Loirand, Stent implantation activates RhoA in human arteries: inhibitory effect of rapamycin, J. Vasc. Res. 42 (2005) 21–28.
[36] N. Sawada, H. Itoh, K. Ueyama, J. Yamashita, K. Doi, T.H. Chun, M. Inoue, K. Masatsugu, T. Saito, Y. Fukunaga, S. Sakaguchi, H. Arai, N. Ohno, M. Komeda, K. Nakao, Inhibition of rho-associated kinase results in suppression of neointimal formation of balloon-injured arteries, Circulation 101 (2000) 2030–2033.
[37] C. Herdeg, M. Fitzke, M. Oberhoff, A. Baumbach, S. Schroeder, K.R. Karsch, Effects of atorvastatin on in-stent stenosis in normo- and hypercholesterolemic rabbits, Int. J. Cardiol. 91 (2003) 59–69.
[38] Z. Mallat, A. Gojova, V. Sauzeau, V. Brun, J.S. Silvestre, B. Esposito, R. Merval, H. Groux, G. Loirand, A. Tedgui, Rho-associated protein kinase contributes to early atherosclerotic lesion formation in mice, Circ. Res. 93 (2003) 884–888.