Increased Expression and Activity of RhoA Are Associated With Increased DNA Synthesis and Reduced p27Kip1 Expression in the Vasculature of Hypertensive Rats

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Abstract—We have previously shown that the function of the small G protein Rho is required for vascular smooth muscle cell proliferation and migration. We hypothesized that changes in Rho or Rho signaling might contribute to enhanced vascular proliferative responses associated with hypertension. Western blot analysis revealed that total RhoA expression was ~2-fold higher in aortas, tail arteries, and aortic smooth muscle cells (ASMCs) obtained from adult male spontaneously hypertensive rats (SHR) compared with those from Wistar Kyoto rats (WKY). An increase in active GTP-bound RhoA was detected in aortic homogenates by affinity precipitation with the RhoA effector rhotekin and by examining RhoA-[35S]GTPγS binding. RhoA protein and activity were also increased in vessels from rats treated with N-nitro-L-arginine methyl ester to increase blood pressure. Thrombin-stimulated RhoA activation was also significantly greater in ASMCs from SHR. As a functional correlate of these changes in Rho signaling, thrombin-stimulated DNA synthesis was enhanced in tail arteries and ASMCs from SHR. Expression of the cyclin-dependent kinase inhibitor p27Kip1 was decreased by two thirds in SHR, and this decrease was mimicked in ASMCs by expression of a constitutively active (GTPase-deficient) mutant of RhoA. Wortmannin (10 nmol/L) fully inhibited the decrease in p27 Kip1 induced by RhoA, and a membrane-targeted catalytic subunit of phosphatidylinositol-3 kinase (PI3K [p110 CAAX ]) decreased p27 Kip1 expression, suggesting that RhoA signals through PI3K. These data provide evidence that RhoA brings about changes in DNA synthesis through reduced expression of p27Kip1, mediated in part via PI3K, and suggest that increases in RhoA expression and activity contribute to the enhanced vascular responsiveness observed in hypertension. (Circ Res. 2001; 89:488-495.)

Key Words: vascular smooth muscle ■ spontaneously hypertensive rats ■ Rho ■ p27Kip1 ■ DNA synthesis

Hypertension is a cardiovascular disorder characterized by altered regulation of vascular tone. Although enhanced vascular contractility is the hallmark of hypertension, smooth muscle cell proliferation1,2 and migration3 are also increased in cells from hypertensive rats. The proliferation and migration of smooth muscle cells from the media to the intima are involved in a variety of vascular disorders and contribute to the pathophysiological progression of atherosclerosis, restenosis, graft rejection, and renal failure. Enhanced neointimal formation has also been observed in vessels from hypertensive rats.4,5 Clinical studies indicate that traditional antihypertensive therapies are inadequate at reducing the coronary events and renal failure associated with hypertension (see review6). Recent work has shown that heterotrimeric GPCRs signal through small G proteins to mediate the aforementioned responses (see reviews7,8). There is an extensive literature documenting the involvement of the small G protein Rho in GPCR-stimulated Ca2+ sensitization of vascular smooth muscle contraction.1,12 Rho, acting through its effector Rho kinase, inhibits myosin phosphatase, favoring accumulation of phosphorylated myosin light chain13 and enhanced contraction. Support for this mechanism includes evidence that the catalytic subunit of Rho kinase leads to contraction of permeabilized vessels,14 whereas the Rho kinase inhibitor Y-27632 blocks agonist-stimulated or guanine nucleotide–stimulated contraction.15 Importantly, Y-27632 has also been shown to reduce blood pressure in three different forms of experimental hypertension in rats.16 These studies highlight the importance of the Rho–Rho kinase pathway in the regulation of blood pressure.

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Recent work from our laboratory has shown that inhibition of Rho or Rho kinase prevents thrombin-stimulated DNA synthesis and migration in aortic smooth muscle cells (ASMCs), providing evidence that these GPCR-stimulated vascular responses are also dependent on the Rho–Rho kinase pathway. In contrast to the abundance of mechanistic data describing the pathway for Rho-mediated contraction, less is known about the downstream effectors of Rho that are involved in cell proliferative responses. Interestingly, in fibroblasts, Rho has been shown to be necessary for Ras-mediated DNA synthesis. We have shown that RhoA synergizes with Ras to stimulate DNA synthesis in rat ASMCs. One mechanism by which Rho regulates cell cycle progression is by reducing the expression of the cyclin-dependent kinase inhibitor (CKI) p27^Kip1. CKIs such as p27^Kip1 and p27^{G1} bind to and inhibit the activity of cyclin-dependent kinases and thereby prevent cell cycle progression. Therefore, a reduction in cellular levels of these CKIs allows cell cycle progression to occur. Of further interest, the cholesterol-lowering agent simvastatin blocks platelet-derived growth factor-mediated proliferation by preventing decreases in the expression of the CKI p27^Kip1. This appears to be explained by the ability of statins to block Rho function by preventing its geranylgeranylation and membrane-targeting.

The present investigation was undertaken to determine whether RhoA, Rho kinase, or Rho signaling pathways were altered in the vasculature of hypertensive rats. Our data demonstrate that there is not only greater RhoA expression but also significantly enhanced RhoA activity in aortas obtained from spontaneously hypertensive rats (SHR) versus Wistar-Kyoto rats (WKY) and in aortas from rats treated with L-NAME. In addition, levels of p27^{G1} are diminished in SHR, suggesting a mechanism for the enhanced DNA synthesis associated with the upregulated RhoA expression/function. The studies also provide evidence of a role for phosphatidylinositol-3 kinase (PI3K) in RhoA-mediated decreases in vascular p27{^G1} expression and cell cycle progression.

Materials and Methods

Removal and Preparation of Aortas

Male WKY and SHR (21 of each strain), aged 12 to 14 weeks, were obtained from Charles River Laboratories (Wilmington, Mass) or provided by Dr Morton Printz (University of California, San Diego, La Jolla, Calif). Rats were killed by decapitation. Aortas were removed from the arch through the abdominal region. Connective tissue was removed by scraping the outer surface of the aorta. Aortas for Western blot analysis were frozen on dry ice and maintained at −80°C until glass-to-glass homogenization. Aortas for measurement of RhoA activation were homogenized on ice, and assays were performed immediately after removal.

Rat ASMCs

ASMCs from adult male Sprague-Dawley rats were obtained as previously described. ASMCs from SHR and WKY were a generous gift from Dr Edwin K. Jackson and Dilbert G. Gillespie, University of Pittsburgh, Pittsburgh, Pa. These cells were cultured as explants from the ascending thoracic aortas, obtained from 16-week-old male SHR and WKY (Taconic Farms, Germantown, NY) as previously described.

Western Blot Analysis

Equal amounts of protein were loaded onto polyacrylamide gels. Proteins were separated, transferred to Immobilon membranes (Millipore), and blocked with 3% BSA at room temperature for 1 hour. Blots were probed with antibodies to RhoA (1:1000), Ras (1:250), p27^{G1} (1:750), p27^{G1} (1:750, Santa Cruz Biotechnology), Rho-GDI (1:500; Zymed), or ROCK (1:1000, Transduction Laboratories) at 4°C overnight. Blots were then incubated with the appropriate horseradish peroxidase–conjugated secondary antibody (1:2000, Sigma Chemical Co) for 1 hour at room temperature. The signal was detected by using enhanced chemiluminescence, and film was analyzed by imaging.

Measurement of RhoA Activation State

Vessels

RhoA-effector binding was determined by using a glutathione S-transferase (GST)-fusion protein of the Rho binding domain (RBD) of the Rho effector rhotekin (a generous gift from Dr Martin Schwartz, The Scripps Research Institute, La Jolla, Calif). Buffers for RhoA-RBD were adapted from Kranenburg et al, as described in our previous work. Aortas were homogenized in complete RBD buffer, spun at 500g for 5 minutes at 4°C. To assay RhoA-[^{35}S]GTP{^γ}S binding, an aliquot (25 mg of protein) from the supernatant was added to complete lysis buffer containing a final concentration of 300 mmol/L [^{35}S]GTP{^γ}S. The remaining supernatant was further centrifuged at 14 000g for 2 minutes at 4°C. Affinity precipitation with the rhotekin RBD and Western blot for RhoA were performed as previously described.

WKY and SHR ASMCs

Cells were split 1:10 and set on 10-cm culture dishes. Five days later, cells were stimulated with either vehicle or agonist for 3 minutes at 37°C. The reaction was stopped with ice-cold Tris-buffered saline solution. Cells were lysed in ice-cold RBD buffer, scraped, placed into a microfuge tube, and centrifuged at 14 000g for 2 minutes at 4°C. Affinity precipitation and Western blotting were performed as previously described.

L-NAME Treatment of WKY

The 12 WKY obtained from Charles River Laboratories were allowed to acclimate for 1 week before treatment with 50 mg/100 mL L-NAME (≈35 mg/kg per day) in their drinking water for 4 weeks. Rats were euthanized, and their aortas were removed as described above. Assay and Western blotting for RhoA and RhoA-RBD were performed as described above.

[^H]Thymidine Incorporation

Tail arteries were cut into 5-mm segments and cultured under ASMC conditions for 3 days. Each segment was placed into the well of a 12-well plate and serum-starved for 24 hours before the addition of either vehicle, thrombin, or 20% serum. [^H]Thymidine incorporation was measured as previously described.

CKI Expression After Adenoviral Infection or Agonist Treatment

ASMCs were plated on 6-cm dishes at 2×10^4 cells/mL and grown for 3 days. Cells were serum-starved overnight and infected with adenoviral constructs encoding either AdCMV, L63RhoA, or the activated p110_{CAAX} (which is the catalytic subunit of PI3K containing a membrane targeting CAAX domain) at 500 viral particles per cell as previously described. This titer of virus gives an infection efficiency of ≈75%, based on infection with LacZ adenovirus and β-galactosidase staining. Twenty-four hours after infection, the cells were washed with serum-free media and harvested 24 hours later, and proteins were Western-blotted for p27^{G1}. For agonist stimulation, 1.5 million cells were set on 6-cm dishes for 8 days, serum-starved for 24 hours, and stimulated with agonist for another 24 hours before harvest.
Statistical Analysis

Data were analyzed by 1-way ANOVA or Student t test where appropriate. For P<0.05, post hoc analysis was performed by using the Tukey-Kramer multiple comparisons test.

Results

DNA Synthesis Is Accentuated in Blood Vessels and ASMCs From SHR

To compare proliferative responses of blood vessels from SHR versus WKY rats, we measured DNA synthesis by using [3H]thymidine incorporation. Both thrombin and serum induced greater [3H]thymidine incorporation in tail arteries from SHR than from WKY (Figure 1A). These findings are consistent with previous reports of increased DNA synthesis in VSMCs from hypertensive rats.1,2 Our earlier studies demonstrated that thrombin-stimulated DNA synthesis in rat ASMCs was dependent on the small G protein Rho.16 To determine whether thrombin-stimulated DNA synthesis of SHR and WKY tail arteries was also Rho dependent, we treated vessels with the C3 exoenzyme from Clostridium botulinum, which selectively ribosylates and functionally inactivates Rho. Treatment with 40 μg/mL C3 exoenzyme for 12 hours resulted in complete inhibition of thrombin-stimulated [3H]thymidine incorporation in tail arteries (data not shown), indicating Rho-dependence.

Blood vessels are composed of a variety of cell types, including endothelial cells, fibroblasts, and VSMCs. To determine whether enhanced thrombin-stimulated DNA synthesis occurred in smooth muscle cells, ASMCs isolated from SHR and WKY were examined. As shown in Figure 1B, thrombin produced a significant increase in [3H]thymidine incorporation in both cell types, but the response was significantly higher in cells from SHR. C3 treatment fully blocked thrombin-stimulated DNA synthesis of ASMCs from SHR and WKY (Figure 1B), also indicating Rho-dependence. The greater thrombin response is not due to an increase in receptors, because Western blot with an antibody to the thrombin receptor, protease activated receptor (PAR1), revealed no difference in expression between aortic homogenates from SHR versus WKY (data not shown).

RhoA Expression Levels Are Higher in Aortas From SHR

To assess differences in RhoA or Rho-dependent signaling pathways in vessels from hypertensive rats, rat aortas and tail arteries were isolated from SHR and WKY, and extracts were prepared for Western blot analysis. Immunoblots with anti-RhoA demonstrated ~2-fold higher levels of RhoA (per mg protein) in aortas from adult SHR than from WKY (Figure 2A). Aortas from 4-week-old prehypertensive SHR also exhibited a significant increase in RhoA expression (per mg protein) (Figure 2A), suggesting that the increase in RhoA is not a result of hypertension. Compared with tail arteries from

Statistical Analysis

Data were analyzed by 1-way ANOVA or Student t test where appropriate. For P<0.05, post hoc analysis was performed by using the Tukey-Kramer multiple comparisons test.
WKY, tail arteries from SHR showed similarly greater RhoA expression (data not shown), indicating that this alteration is not specific to the aorta. In contrast, the expression of Ras, another small G protein, was similar in aortic homogenates from SHR and WKY (Figure 2B). Thus, greater RhoA expression does not represent a generalized increase among all small G proteins. Rho has been shown to signal through its effector Rho kinase/ROCK to mediate VSMC contraction, DNA synthesis, and migration. There was no difference in the level of expression of ROCK in aortic homogenates from SHR and WKY (Figure 2C). Because the fraction of RhoA in the cytosol was also increased in SHR (see below), we examined changes in the level of expression of Rho-GDI, which localizes Rho to the cytosol and prevents GDP dissociation. However, the expression of Rho-GDI was not different in aortic homogenates from WKY versus SHR (Figure 2D).

**RhoA Activity Is Greater in Aortas From SHR**

Rho function depends on its conversion from the GDP- to the GTP-bound state. The extent of RhoA activation was assessed by a pull-down assay in which a GST fusion protein of the RBD of the Rho effector rhotein is used to affinity-precipitate the active (GTP-ligated) RhoA. Specificity of this assay for active RhoA was confirmed in our previous work. As shown in Figure 3A, a significantly greater fraction of total RhoA was activated in aortic homogenates from SHR versus WKY. An assay that we developed earlier assessed RhoA-[35S]GTPγS binding, taking advantage of the increased ability of activated G proteins to release GDP in exchange for radiolabeled GTP. Using this assay, we also observed increases in activated RhoA in aortic preparations from SHR versus WKY rats (Figure 3B). Increases in membrane-associated RhoA have been widely used as an indicator of increases in active RhoA. Surprisingly, the fraction of RhoA associated with the cytosol, rather than that associated with the membrane, increased in SHR (data not shown), suggesting dissociation between RhoA membrane association and its activation state.

**RhoA Expression and Activity Are Greater in ASMCs From SHR**

To demonstrate that alterations in RhoA expression and activity occurred in VSMCs, we examined ASMCs from SHR and WKY. The level of RhoA expression per mg protein was ~2-fold higher in cells isolated from SHR than in cells isolated from WKY (Figure 4A), in concordance with observations made with the use of vessel homogenates (Figure 2A). The activity of RhoA assessed by RhoA-RBD binding was not different in unstimulated (control) cells from SHR and WKY rats (Figure 4B). However, stimulation with thrombin induced marked activation of RhoA in ASMCs, and this response was significantly greater in SHR than in WKY cells (Figure 4B).
RhoA Expression and Activity Are Elevated in Aortas From L-NAME–Treated Rats

An important question arising from the present study is whether increases in RhoA expression and activity are specific for the SHR model or characteristic of other models of hypertension. We chose an established model of NO-deficient hypertension in which WKY rats were administered L-NAME (50 mg/100 mL or $35 \text{ mg/kg per day}$) in their drinking water for 4 weeks. Western blot analysis revealed that aortas from L-NAME–treated rats had a higher expression of RhoA per mg protein than did aortas from untreated WKY (Figure 5A). Aortas from L-NAME–treated rats had a corresponding increase in RhoA activity assessed by RhoA-RBD binding (Figure 5B). These data demonstrate that increases in RhoA expression and activity are not unique to vessels from SHR and may therefore represent a vascular alteration associated with hypertension.

Expression of the Cell Cycle Inhibitor p27 Kip1 Is Reduced in Aortas From SHR

One mechanism believed to underlie the effect of Rho on cell DNA synthesis is decreased expression of CKIs, such as p21Waf1/Cip1 and p27 Kip1.17,18 Western blots of aortic homogenates from WKY and L-NAME–treated rats showed that total RhoA per mg protein is higher in the L-NAME group ($P<0.01$). B, RhoA-RBD binding was also significantly greater in aortic homogenates from L-NAME–treated rats ($P<0.01$). Data represent mean±SE from 4 to 6 rats per group.

Adenoviral Expression of GTPase-Deficient RhoA Reduces Expression of p27 Kip1 but not p21 Waf1/Cip1

The data above suggested that the increased RhoA expression/activity could be responsible for decreases in p27 Kip1 expression. To determine whether RhoA activation was sufficient to reduce levels of p27 Kip1, ASMCs were infected with GTPase-deficient activated RhoA (L63RhoA) in adenovirus. The expression of p27 Kip1 was significantly reduced by the expression of L63RhoA (Figure 7A). In contrast, L63RhoA expression did not alter p21 Waf1/Cip1 expression (Figure 7A).

Involvement of PI3K in RhoA-Mediated Decrease in p27 Kip1

Because PI3K has been implicated in the regulation of p27 Kip1,25 we asked whether the inhibitory effect of RhoA was mediated via PI3K. ASMCs were first infected with p110Caax, the catalytic subunit of PI3K containing a CAAX domain targeting it to the plasma membrane. Infection with

**Figure 5.** RhoA expression and activity are increased in aortas from L-NAME–treated rats. A, Western blot analysis of aortic homogenates from WKY and L-NAME–treated rats showed that total RhoA per mg protein is higher in the L-NAME group ($P<0.01$). B, RhoA-RBD binding was also significantly greater in aortic homogenates from L-NAME–treated rats ($P<0.01$). Data represent mean±SE from 4 to 6 rats per group.

**Figure 6.** Reduced expression of the CKI p27 Kip1, but not p21 Waf1/Cip1, in aortas from SHR. Western blot analysis of aortic homogenates with use of an antibody to p27 Kip1 (A) revealed a doublet band at the expected molecular weight (inset: W indicates WKY; S, SHR). The upper band was not significantly different in aortic homogenates between strains (data not shown). The lower band was significantly decreased in homogenates from SHR ($P<0.01$). Western blot with an antibody to p21 Waf1/Cip1 (B) showed no difference in aortic homogenates from SHR vs WKY rats. Data represent mean±SE from 7 aortas from each strain.
p110CAAX significantly decreased the expression of p27Kip1 (Figure 7B). To determine whether PI3K acts downstream of RhoA to mediate decreases in the expression of p27Kip1, rat ASMCs were treated with 10 nmol/L wortmannin before infection with L63RhoA. The L63RhoA-induced decrease in p27Kip1 was abolished (Figure 7B). These data suggest an involvement of PI3K in the RhoA-mediated reduction in p27Kip1 expression. Another PI3K inhibitor, LY294002 (30 μmol/L), likewise attenuated the L63RhoA-induced decrease in p27Kip1 but had deleterious effects on cell viability, limiting the interpretation of data obtained with this inhibitor (data not shown).

Thrombin-Stimulated DNA Synthesis Is Associated With Decreases in p27Kip1 and Blocked by Wortmannin

We have previously reported that thrombin stimulates Rho-dependent [3H]thymidine incorporation in rat ASMCs. The data presented above suggest that thrombin and Rho may bring about these changes via a PI3K-mediated reduction in p27Kip1. To determine the involvement of PI3K in thrombin-stimulated DNA synthesis, we treated cells with 10 nmol/L wortmannin, which fully blocked thrombin-stimulated [3H]thymidine incorporation (Figure 8A). The possibility that thrombin exerts its effects on DNA synthesis in ASMCs by decreasing the expression of p27Kip1 was also examined. As shown in Figure 8B, thrombin produced a marked decrease in p27Kip1 protein levels. Significantly, phenylephrine, a GPCR agonist that we previously reported to neither activate RhoA nor induce DNA synthesis in rat ASMCs, failed to decrease p27Kip1 expression.

Discussion

Hypertension is accompanied by enhanced vascular contractility. The observations that agonist-stimulated (but not KCl-stimulated) arterial contraction is enhanced in SHR and that contraction of arteries at constant pCa is increased in vessels from SHR suggest that Rho-mediated Ca sensitization may be enhanced in hypertension. This is further substantiated by observations that the Rho kinase inhibitor Y-27632 produced a more pronounced vasodilator response in cerebral vessels from hypertensive rats and reduced hypertension in three experimental models. VSMC proliferation and migration are likewise increased in hypertension. In particular, VSMCs from hypertensive rats exhibit a greater ability to proliferate in culture when grown in serum or stimulated with angiotensin II. Vascular smooth muscle and endothelial cells from hypertensive rats also possess an increased migratory capacity compared with that of cells from normotensive rats, paralleling the capacity of vessels from hypertensive rats toward greater neointimal forma-

Figure 7. Levels of p27Kip1 are reduced by RhoA (L63RhoA) and PI3K (p110CAAX). A, Infection of ASMCs from Sprague-Dawley rats with adenoviral constructs encoding for L63RhoA resulted in a significant decrease in p27Kip1, but not p21Waf1/Cip1, expression. B, Adenoviral infection with p110CAAX produced a significant decrease in p27Kip1 expression. Wortmannin (Wort, 10 nmol/L) was added to ASMCs 1 hour before adenoviral infection and again 24 hours later. The L63RhoA-mediated decrease in p27Kip1 was fully inhibited by Wort treatment. There was no difference between AdCMV in the presence or absence of Wort. Data represent mean±SE from 3 or 4 experiments performed in duplicate or triplicate. *P<0.05 compared with AdCMV; #P<0.05 compared with L63RhoA alone.

Figure 8. Thr stimulates DNA synthesis and decreases levels of p27Kip1. A, Wort (10 nmol/L) was added to ASMCs from Sprague-Dawley rats 1 hour before agonist and again 24 hours later. Thr significantly increased [3H]thymidine incorporation in the absence of Wort (*P<0.001 compared with Ctr) but not in the presence of Wort (#P<0.001 compared with Thr alone). Data represent mean±SE from 3 experiments, with each performed in duplicate or triplicate. B, Stimulation with Thr, but not phenylephrine (PE), produced a decrease in p27Kip1 in ASMCs. Data are from 1 experiment performed in triplicate.
tion,4,5 Thus, these Rho-mediated responses also appear to be increased in association with hypertension.

We hypothesized that Rho or Rho-signaling pathways were increased in the vasculature of hypertensive animals. The data reported in the present study support this hypothesis, demonstrating a higher level of expression of RhoA in the vasculature of SHR and L-NAME–treated rats versus WKY. RhoA expression is greater in aortas and in tail arteries from SHR, indicating that this alteration is not specific to one vessel type. RhoA expression was also greater in ASMCs from SHR versus WKY, demonstrating that the increase in RhoA observed in the vasculature of SHR occurs in the smooth muscle cells. The finding that differential expression of RhoA is conserved even after isolation, culture, and passage of ASMCs from SHR suggests that this is, at least in part, an autonomous alteration, independent of stretch or humoral changes accompanying hypertension. Our observation that RhoA expression is also increased 1.6-fold in aortas from SHR at 4 weeks, an age before the onset of hypertension, likewise suggests that RhoA upregulation may be independent of increases in blood pressure.

A key finding in the present study is that the amount of activated RhoA also increased in vessels from SHR and L-NAME–treated rats. In ASMCs isolated from SHR, basal RhoA activity was not increased, but thrombin-stimulated activation of RhoA was significantly enhanced. Taken together, these findings suggest that VSMCs from hypertensive rats have an increased ability to respond to chemical or physical stimuli by activating RhoA, which in turn provides a potential mechanism for the enhanced Rho-dependent agonist responsiveness of the SHR vasculature.

Our finding that thrombin and serum caused greater DNA synthesis in vessels and ASMCs from hypertensive rats is consistent with previous reports of increased VSMC proliferation in hypertension.1,2 Data in the present study extend these findings and provide insight into a potential mechanism for this observation. Vessels from hypertensive rats contain more RhoA and an increase in activated RhoA. Although we have previously shown that overexpression of activated RhoA alone is not sufficient to induce DNA synthesis in rat ASMCs, we have demonstrated that RhoA synergizes with and enhances Ras-mediated DNA synthesis.16 Similar data from fibroblasts show that Rho complements Ras-mediated cell cycle progression by reducing the expression of p21Waf1/Cip1.17 Consistent with these observations, we observed less p27Kip1 in aortas from SHR. By overexpressing GTPase-deficient activated L63RhoA in rat ASMCs derived from normotensive rats, we were able to induce significant decreases in the expression of p27Kip1, mimicking those observed in vessels from hypertensive rats.

Our results also provide evidence for the involvement of PI3K in the RhoA-mediated reduction of p27Kip1 levels associated with cell cycle progression. Expression of either GTPase-deficient L63RhoA or membrane-localized PI3K reduced cellular levels of p27Kip1. Additionally wortmannin and LY294002 prevented the decrease in p27Kip1 induced by L63RhoA, suggesting PI3K as a downstream mediator of the effects of RhoA. Our results bring together previous results of others who have suggested that RhoA can signal through PI3K11 and that PI3K can regulate p27Kip1 expression.25 The mechanism(s) by which Rho and PI3K regulates p27Kip1 expression is currently under investigation. One report suggests that PI3K acts through the Akt/protein kinase B pathway to promote phosphorylation of a known transcription factor (AFX) and to inhibit its ability to transactivate p27Kip1.25 Alternative possibilities are that Rho and/or PI3K use as-yet-unknown mechanisms to increase phosphorylation, ubiquitination, and subsequent degradation of p27Kip1.1,2

In summary, the data in the present study provide evidence for increased vascular RhoA expression and activity in hypertension that would contribute to the enhanced Rho-mediated responses observed in vessels and VSMCs from hypertensive animals. The decrease in p27Kip1 expression in aortas from SHR and the ability of activated RhoA to decrease p27Kip1 in ASMCs suggest that increased RhoA activity in the hypertensive vasculature may serve as a trigger for decreases in p27Kip1 and concomitant increases in cell cycle progression.

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