Angiotensin II induces RhoA activation through SHP2-dependent dephosphorylation of the RhoGAP p190A in vascular smooth muscle cells

Jeremy Bregeon,1,2 Gervaise Loirand,1,2,3 Pierre Pacaud,1,2 and Malvyne Rolli-Derkinderen1

1Institut National de la Santé et de la Recherche Médicale, UMR915, l’institut du thorax, Nantes; 2Université de Nantes, Nantes; 3Centre Hospitalier Universitaire Nantes, Nantes, F-44000 France

Submitted 23 April 2009; accepted in final form 13 August 2009

Bregeon J, Loirand G, Pacaud P, Rolli-Derkinderen M. Angiotensin II induces RhoA activation through SHP2-dependent dephosphorylation of the RhoGAP p190A in vascular smooth muscle cells. Am J Physiol Cell Physiol 297: C1062–C1070, 2009. First published August 19, 2009; doi:10.1152/ajpcell.00174.2009.—Angiotensin II (ANG II) is a major regulator of blood pressure that essentially acts through activation of ANG II type 1 receptor (AT1R) of vascular smooth muscle cells (VSMC). AT1R activates numerous intracellular signaling pathways, including the small G protein Rho A known to control several VSMC functions. Nevertheless, the mechanisms leading to RhoA activation by AT1R are unknown. RhoA activation can result from activation of RhoA exchange factor and/or inhibition of Rho GTPase-activating protein (GAP). Here we hypothesize that a RhoGAP could participate to RhoA activation induced by ANG II in rat aortic VSMC. The knockdown of the RhoGAP p190A by small interfering RNA (siRNA) abolishes the activation of RhoA-Rho kinase pathway induced after 5 min of ANG II (0.1 μM) stimulation in rat aortic VSMC. We then show that AT1R activation induces p190A dephosphorylation and inactivation. In addition, expression of catalytically inactive or phosphoresistant p190A mutants increases the basal activity of RhoA-Rho kinase pathway, whereas phosphomimetic mutant inhibits early RhoA activation by ANG II. Using siRNA and mutant overexpression, we then demonstrate that the tyrosine phosphatase SHP2 is necessary for 1) maintaining p190A basally phosphorylated and activated by the tyrosine kinase c-Abl, and 2) inducing p190A dephosphorylation and RhoA activation in response to AT1R activation. Our work then defines p190A as a new mediator of RhoA activation by ANG II in VSMC.

RhoA; phosphorylation; SH2-containing protein tyrosine phosphatase 2; Abelson tyrosine kinase

The hemodynamic abnormalities observed in hypertension are influenced by many humoral factors, among which angiotensin II (ANG II) seems to be critical (for review see Ref. 43). ANG II acts through two distinct subtypes of receptors, type 1 (AT1R) and type 2 (AT2R), that produce opposite effects on vascular functions. The G protein-coupled AT1R mediates the known physiological and pathological actions of ANG II and undergoes rapid desensitization and internalization after agonist stimulation. At the cellular level, ANG II stimulates vascular smooth muscle cell (VSMC) growth and hypertrophy, increases collagen deposition, induces inflammation, increases contractility, and decreases dilation. Molecular mechanisms associated with these changes include upregulation of many signaling pathways, including tyrosine kinases, mitogen-activated protein kinases, generation of reactive oxygen species, and activation of the small G protein RhoA and its target Rho kinase (ROCK) (19, 42). The activation of the RhoA-ROCK pathway is responsible for both vasoconstriction and vascular remodeling (22, 30). RhoA-ROCK plays an important role in mediating various VSMC functions, such as contraction through calcium-sensitization of contractile proteins, differentiation, proliferation, cell adhesion and motility, cytokinesis, and expression of genes involved in vascular remodeling (21, 22). However, until now, the molecular mechanisms responsible for activation of the RhoA-ROCK pathway by AT1R receptor stimulation by ANG II in VSMC remains poorly understood.

RhoA acts as molecular switch, the activity of which is under the direct and tight control of regulatory proteins (6, 8). In the inactive GDP-bound form, Rho is locked in the cytosol by guanine dissociation inhibitors. The guanine nucleotide exchange factors (GEFs) catalyze the exchange of GDP for GTP to activate RhoA (32). In the active GTP-bound form, Rho translocates to plasma membrane where it interacts with effectors to transduce the signal downstream. GTPase-activating proteins (GAPs) hydrolyze GTP to GDP then turn off activation. Among the dozen of GAPs encoded by vertebrate genomes with predicted activity toward Rho proteins, five preferentially inhibit RhoA: p50, Myr5, GRAF, and two highly homologous RhoGAPs, p190A, and p190B (36). The p190 GAPs are the most studied GAPs because they are widely expressed and potent regulators of several of the Rho proteins. The p190 RhoGAPs undergo complex regulation, including phosphorylation, interaction with other proteins or phospholipids, subcellular translocation, and proteolytic degradation (3). p190A is known to be regulated by integrins, and its activity is required to downregulate RhoA during cell-matrix adhesion (2), cytokinesis (38), or cell-cell adhesion and motility (1, 4, 5). However, the mechanisms that regulate RhoGAPs and their involvement in the activation of RhoA-ROCK in response to G protein-coupled membrane receptor stimulation remain unclear.

The aim of the present research is thus to determine whether RhoGAPs are involved in the activation of RhoA-ROCK pathway by ANG II in VSMC. In a first set of experiments, we identified the RhoGAP p190A as critical in the early activation of RhoA-ROCK by ANG II. In the second part of this study, we showed that the tyrosine phosphatase SHP2 tightly controls p190A phosphorylation and thus RhoA-ROCK activation by ANG II.

Materials and Methods

Cell culture, transfection, and treatment. Rat aortic smooth muscle cells (RASMC) were obtained from explant culture. Only smooth muscle cells at passage 3 were used in this study. They were plated at 70–80% confluence for cDNA transfection using the Nucleofector.
p190A is involved in Ang II-induced RhoA activation in RASMC

C1063

(LONZA/Amaza) according to the manufacturer’s instructions. Briefly 2 × 10⁶ cells were electroporated with 4 μg of plasmid using the D33 program and replated in DMEM medium containing 10% fetal calf serum for 24 h. Small interfering RNA (siRNA) were transfected with jetPEI (Qbiogen, Illkirch, France) according to the manufacturer’s instructions. Transfected or untransfected RASMC were serum starved for the following 24 h and treated with Ang II (100 nM) for the indicated time in the presence of PD-123319 (10 μM) to inhibit AT2 receptors in all experiments except those in Fig. 3, C and F, where they are pretreated with ATIR inhibitor losartan (10 μM), and those in Fig. 3, A and D, with no pretreatment.

siRNA. The sense and antisense strands of siRNAs (Eurogentec, Seraing, Belgium) were: the following: GGRA, sense 5′-GCCGAGAGAGGGAAGCGG-3′, antisense 5′-AUUCUGACAAAGGAUCCCG-3′; p190AngII-GAP, sense 5′-GACCUUGUCACUGAACTGGA-3′, antisense 5′-AGUAUAAGAGGGAGGUGCU-3′; p50RhoGAP, sense 5′-GAACUGGUGGCUCCUAAGAAGA-3′, antisense 5′-UUCAGACAGAGGGACGUCAU-3′; p50RhoGAPsH2, sense 5′-UUGAGUGUCCUAGGUGCAU-3′, antisense 5′-CACCCUAAUAGGACUAAUCUAAU-3′.

The effect of siRNA on transcript level was analyzed by quantitative PCR (Bio-Rad) using the following primers: GAPDH, 5′-CCATGCATTGCGCTACT-3′, down 5′-GTCATCATCTGAGCAGGTTC-3′; GGRA, 5′-GCGACTCATCCGCACTATCA-3′, down 5′-GCAAGTTTGAGGCGAGG-3′, Myr5, 5′-CCAGCGGTCAGTTCGAC-3′, down 5′-TCTCTACCTCCACAGCA-3′; p190RhoGAP-A, 5′-CTCCTCTGCTTGTTGATG-3′, antisense 5′-GCCUGGAGCCGAGCACAACG-3′; Scrambled, sense 5′-GCGUGAGCAGACUCAUTC-3′; Shp2, sense 5′-UUGAGUGUCCUAGGUGCT-3′, antisense 5′-CACCACUGAGAACUAACAACAT-3′.

p190A is necessary to maintain low basal RhoA-ROCK activity and to induce early RhoA activation by Ang II. To analyze the role of GAPS in RASMC, we have designed siRNA targeting the rattus norvegicus sequences of p50, Myr5, GGRA, and p190A GAPS. All siRNAs significantly reduced specifically their targeted GAP mRNA (Fig. 1A). To confirm the silencing of protein expression by siRNA, immunoblots were performed and showed that p190A protein expression was decreased to 0.28 ± 0.09 (Fig. 1B). Unfortunately there were no commercially available antibodies suitable for a similar analysis of p50RhoGAP, Myr5, or GGRA (data not shown). We have previously shown that stimulation of RASMC by Ang II for 2–240 min induced two distinct peaks of RhoA-ROCK activation occurring after 5 and 60 min of stimulation (14). Therefore, we have measured RhoA-ROCK pathway activation at these two times by the phosphorylation loading was checked by reprobing the membrane with monoclonal anti-α-tubulin antibody.

Immunoprecipitation and communoprecipitation. Immunoprecipitations were carried out with monoclonal anti-p190A antibody (1/500) or anti-Abelson tyrosine kinase (cAb1) antibody (1/500) and G-Sepharose beads (fast-flow; General Electric) on a rotating wheel overnight at 4°C. The protein G-Sepharose-bound immune complexes were washed twice in NETF buffer containing Nonidet P-40 (1% wt/vol) and once in NETF without detergent. Pellets from the immunoprecipitations were heated at 95°C for 10 min in Laemmli sample buffer for SDS-polyacrylamide gel electrophoresis and analyzed by immunoblot.

Measurement of MYPT phosphorylation and RhoA activity. ROCK target of RhoA is a Ser/Thr kinase that acts on vessels contraction by phosphorylating the Thr696 of the myosin phosphatase target protein-1 (MYPT1). Measurement of MYPT Thr696 phosphorylation by immunoblotting with a specific phospho-Thr696 was used to analyze the activation of RhoA-ROCK pathway. The quantification of MYPT phosphorylation was allowed by addition to MYPT expression measured with anti-MYPT1 (H130) before being represented relative to the nonstimulated condition taken as 1. Direct RhoA activation was measured by G-LISA assay (cytoskeleton) according to the manufacturer’s instructions. Briefly, the RhoA G-LISA kit used 96-well plates coated with the Rho-binding domain of the RhoA effector Rhotekin. Rho-GTP was removed during washing steps and Rho-GTP was detected with a RhoA-specific antibody and chemiluminescence. The level of RhoA activation was expressed relative to its basal level, in the absence of Ang II.

Chemicals and drugs. Mouse monoclonal anti-phosphotyrosine antibody (4G10) was purchased from Upstate Biotechnology (Lake Placid, NY). Mouse monoclonal anti-p190A antibody was purchased from BD Transduction Laboratories. Rabbit polyclonal anti-cAbl (C91), anti-SHP2, anti-phospho-MYPT (Thr696; sc-17556), anti-MYPT1 (H130), anti-ERK1 (C-16), and anti-ERK2 (C-14) antibodies were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Mouse monoclonal anti-(p)-42/44 MAPK (Thr202/Tyr204; E10) antibody was purchased from Cell Signaling Technology. Mouse monoclonal anti-α-tubulin (DM 1A) and anti-β-actin (AC-74) antibodies were purchased from Sigma-Aldrich, Ang II, PD-123319, and losartan were purchased from Sigma-Aldrich (Saint-Quentin Fallavier, France). STI571 (Imatinib) was dissolved in a water-propylene glycol (50:50) solution and kept at 4°C until used.

Statistics. All results are expressed as the means ± SD of sample size n. Statistical analyses were performed with Student’s t-test (Fig. 1A), one-way ANOVA (Fig. 2) or two-way ANOVA (others). A value of P < 0.05 or less was considered to be statistically significant and is specified for each experiment in the figure legend.

RESULTS

p190A is necessary to maintain low basal RhoA-ROCK activity and to induce early RhoA activation by Ang II. To analyze the role of GAPS in RASMC, we have designed siRNA targeting the rattus norvegicus sequences of p50, Myr5, GGRA, and p190A GAPS. All siRNAs significantly reduced specifically their targeted GAP mRNA (Fig. 1A). To confirm the silencing of protein expression by siRNA, immunoblots were performed and showed that p190A protein expression was decreased to 0.28 ± 0.09 (Fig. 1B). Interestingly there were no commercially available antibodies suitable for a similar analysis of p50RhoGAP, Myr5, or GGRA (data not shown). We have previously shown that stimulation of RASMC by Ang II for 2–240 min induced two distinct peaks of RhoA-ROCK activation occurring after 5 and 60 min of stimulation (14). Therefore, we have measured RhoA-ROCK pathway activation at these two times by the phosphorylation

AJP-Cell Physiol • VOL 297 • NOVEMBER 2009 • www.ajpcell.org

Downloaded by 10.220.33.3 at April 12, 2017 from http://ajpcell.physiology.org/
of MYPT and by direct assessment of RhoA activity. In RASMC transfected with scrambled siRNA, treatment with ANG II for 5 and 60 min increased significantly MYPT phosphorylation to a 5.6 ± 0.2- and 6.2 ± 0.8-fold of control, respectively, as well as RhoA activity to a 1.56 ± 0.03- and 1.51 ± 0.01-fold of control, respectively. siRNAs targeting p50, Myr5, GRAF, or p190A GAP increased the basal MYPT phosphorylation (3.5 ± 0.6-, 4.2 ± 0.7-, 4.1 ± 1.8-, and 4.9 ±
0.4-fold, respectively) as well as the basal RhoA activity (1.27 ± 0.08-, 1.13 ± 0.03-, 1.21 ± 0.04-, 1.27 ± 0.0007-fold, respectively). Remarkably, only siRNA targeting p190A prevented the early MYPT phosphorylation as well as the RhoA activation induced by a 5-min treatment with ANG II. The delayed activation of RhoA-ROCK observed at 60 min of ANG II stimulation was not affected by all of the four siRNAs (Fig. 1, C and D). Thus all four GAPs are involved in maintaining a low RhoA-ROCK activity in basal conditions in RASMC, but only p190A participates to the early activation of the pathway by ANG II. To check that the effect of siRNAs on RhoA-ROCK pathway was specific and not due to a nonspecific inhibition of other signalings downstream of AT1R, we analyzed the ERK activation, known to depend on Gq (27, 28). ERK1/2 phosphorylation was increased after 5 or 60 min of ANG II treatment but none of the siRNAs did modify this profile (Fig. 1C), demonstrating again that the effects of siRNAs targeting RhoGAPs were specific of the RhoA-ROCK pathway.

AT1 receptor activation induces p190A activation and dephosphorylation. We next performed pull-down assays to assess the catalytic activity of p190A toward RhoA (10). Briefly, RASMC lysates were pulled down with WT-RhoA and the GAP-trap mutant Q63L-RhoA, separated by electrophoresis and blotted with an antibody against p190A (Fig. 1E). The amount of p190A bound to WT-RhoA and Q63L-RhoA was similar between cells treated with ANG II for 60 min and control cells. In contrast, 5-min treatment with ANG II decreased the association between endogenous p190A and WT-RhoA (0.6 ± 0.08-fold, n = 3) or RhoA-Q63L (0.3 ± 0.06-fold, n = 3), indicative of a decreased p190A activity (Fig. 1E). This suggests that p190A permanently exerts its GAP activity on RhoA and that ANG II transiently lowers it.

The catalytic activity of p190A depends, at least in part, on its phosphorylation level on tyrosine (31). We thus analyzed tyrosine phosphorylation of p190A in RASMC. In these cells, p190A was basally phosphorylated on tyrosine (Fig. 2A); treatment with ANG II for times ranging from 2 to 60 min induced a decrease in the phosphorylation of p190A with a maximum drop at 5 min [0.71 ± 0.02-fold (n = 5); Fig. 2A]. This ANG II-induced p190A dephosphorylation was enhanced in the presence of the AT2R antagonist PD-123319, decreasing to 0.3 ± 0.05-fold (n = 8), the basal one at 5 min (Fig. 2B). On the contrary, ANG II-induced p190A-dephosphorylation was abolished in the presence of the AT1R inhibitor losartan (Fig. 2C). As a mirror image of p190 phosphorylation, the early activation of the RhoA-ROCK pathway induced by ANG II stimulation was enhanced in the presence of PD-123319 and was completely abolished in the presence of losartan (14). These results indicate that AT1R activation by ANG II in RASMCs is responsible for early dephosphorylation of p190A and RhoA-ROCK activation and that AT2R opposes these effects.

AT1-induced p190A dephosphorylation triggers RhoA-ROCK activation. To analyze whether the dephosphorylation of p190A by AT1R stimulation was responsible for early RhoA-ROCK activation, we expressed WT-p190A, GAP-deficient R1283A-p190A, phosphoresistant Y1105F-p190A, or phosphomimetic Y1105D-p190A mutants in RASMC (Fig. 3). The activation of RhoA-ROCK induced by ANG II at 5 min in RASMC expressing WT-p190A (5.22 ± 0.05-fold, n = 3, P < 0.05) was similar to that observed in control (mock transfected, 3.32 ± 0.05-fold, n = 3, P < 0.05) cells. Expression of the GAP-deficient R1283A-p190A and the phosphoresistant mutant Y1105F-p190A increased basal RhoA-ROCK activity (6.38 ± 0.87- and 8.64 ± 0.82-fold, respectively, n = 3, P < 0.05) and prevented ANG II-induced activation (6.38 ± 0.87- vs. 5.42 ± 0.65-fold and 8.64 ± 0.82- vs. 8.88 ± 1.24-fold, respectively, n = 3). In contrast, the phosphomimetic mutant Y1105D-p190A decreased the basal level of RhoA-ROCK activity (0.34 ± 0.17-fold, n = 3, P < 0.05) and did not allow further activation (0.33 ± 0.16- vs. 0.34 ± 0.17-fold, n = 3) by stimulation by ANG II (Fig. 3). Again, expression of p190A mutants did not alter the ERK1/2 pathway activation by ANG II, confirming that mutants specifically affect RhoA-ROCK pathway. Altogether these results provide evidence that transient p190A dephosphorylation is responsible for the early RhoA-ROCK activation following AT1R stimulation by ANG II in RASMCs. We therefore sought to identify the molecular mechanism(s) coupling AT1R to p190A dephosphorylation.

**SH2 controls p190A phosphorylation level by recruiting cAbl tyrosine kinase.** The GAP p190A can be phosphorylated either by Src or cAbl family tyrosine kinases (15, 17) and...
dephosphorylated by various tyrosine phosphatases (26, 33). As the tyrosine phosphatase SHP2 is involved in the signaling of ANG II downstream to AT1R (24), we first assessed the effect of siRNA targeting SHP2 mRNA. Surprisingly, siRNA-mediated SHP2 silencing led to p190A dephosphorylation in basal condition and completely prevented further ANG II-induced dephosphorylation (Fig. 4A). Accordingly, the level of RhoA-ROCK activity was basally higher in RASMC treated with SHP2 siRNA and was not increased by ANG II (Fig. 4B). Western blot analysis with anti-SHP2 antibody indicated that under resting condition, SHP2 coimmunoprecipitated with p190A and that SHP2/p190A association was not modified following treatment with ANG II (Fig. 4A). These results suggest that SHP2 positively regulates p190A phosphorylation, possibly through the regulation of a p190A kinase. To address this issue, we first studied the presence of Src and/or c-Abl in the SHP2/p190A protein complex by Western blot analysis. Whereas Src binding was undetected (data not shown), cAbl was bound to p190A both in basal and stimulated condition. The decrease of p190A phosphorylation induced by siRNA-mediated SHP2 silencing was associated with the complete loss of cAbl among proteins coimmunoprecipitated with p190A (Fig. 4A). This indicates that SHP2 is necessary to localize cAbl and p190A in a same protein complex and suggests that p190A phosphorylation is catalyzed by cAbl. To confirm this we next used the cAbl inhibitor STI571 (10 μM). As expected, STI571 inhibited p190A phosphorylation and increased RhoA-ROCK activity under basal condition (Fig. 4, C and D). In the presence of STI571, ANG II had no effect on p190A phosphorylation and did not modify RhoA-ROCK activity at 5 min (Fig. 4, C and D). Taken as a whole, these results strongly suggest that SHP2 acts as a scaffold protein to target cAbl to p190A thereby promoting p190A phosphorylation by c-Abl. SHP2 activity is necessary to dephosphorylate p190A in response to ANG II stimulation. Eventually, beyond its role as a scaffold protein, we aimed at determine whether SHP2 phosphatase is also able to dephosphorylate p190A. To directly analyze SHP2 activity on p190A, we expressed the wild-type (WT-SHP2) or a dominant negative form (C/S-SHP2) of SHP2 in RASMC. WT-SHP2 expression did not change the basal level of p190A phosphorylation or its dephosphorylation induced by ANG II stimulation (Fig. 5A). In contrast, C/S-SHP2 expression inhibited AT1R-induced p190A dephosphorylation observed at 5 min of stimulation with ANG II (Fig. 5A). MYPT phosphorylation was not affected by SHP2 overexpression (WT-SHP2) but surprisingly the dominant negative form (C/S-SHP2) of SHP2 induced MYPT-phosphorylation (Fig. 5A). The abolition of ANG II-induced p190A dephosphorylation in cells expressing C/S-SHP2 occurs without alteration of cAbl activity that is still stimulated by ANG II, similarly to control (Fig. 5B). These results thus show that the catalytic activity of SHP2 is responsible for ANG II-induced p190A dephosphorylation by a direct action on p190A, and that SHP2 is necessary...
to accurately control RhoA-ROCK pathway activation by ANG II.

**DISCUSSION**

Our work demonstrates for the first time that inhibition of p190A RhoGAP is responsible for the early activation of RhoA by ANG II in RASMC. We bring to light the major role of the phosphatase SHP2 in this mechanism. SHP2 regulates p190A phosphorylation by both 1) targeting the tyrosine phosphatase cAbl to p190A and 2) inducing p190A dephosphorylation upon AT1R stimulation (Fig. 6).

By stimulating the intrinsic GTPase activity of Rho proteins, GAP switch off the Rho cycle (40). Consistent with this function, they limit Rho protein signaling in time or location (1). Indeed, the current dogma proposes that Rho protein activation in response to various stimuli is triggered by activation of GEFs. This widely held view essentially relegates the GAPs to a secondary role that is seemingly less significant than that of GEFs, which have received considerably more attention in the context of Rho protein regulation. However, some studies have indicated that, in the absence of extracellular stimuli, inhibition of RhoGAP activity can be sufficient to promote activation of Rho proteins and a consequent Rho-mediated biological response (46). Our work demonstrates that GAPs exert a continuous inhibition on RhoA-ROCK pathway in VSMC. All the RhoGAP siRNAs used, designed to specifically inhibit p190A or p50, Myr5, and GRAF, increase the basal RhoA-ROCK pathway activity without any other stimuli. This again addresses the question of GAP specificity that could be answered if we consider that each GAP exerts its activity not in a substrate-specific manner but in a specific subcellular domain manner (1, 44). Indeed, fractionation analysis have shown that Myr5 is bound to the myosin, whereas GRAF is located in focal adhesion and p190A in cellular protrusions.
In agreement with these data, we observed that a wild-type p190A expressed in resting RASMC was mainly cytosolic, whereas a dominant-negative R1283A-p190A mutant relocated to protrusions (unpublished observation).

Whereas several GAPs control the basal RhoA-ROCK activity, only p190A is involved in the early RhoA-ROCK activation induced by ANG II. Although some papers already tried to understand how ANG II could regulate p190A, they led to contradictory results. ANG II was found to induce phosphorylation and thereby stimulation of p190A to limit Rho activity in smooth muscle (34), whereas it did not modify p190 phosphorylation in cardiomyocytes (7). In sphincteric smooth muscles, ANG II-induced contraction was attenuated by antibodies against p190 (29). We show here that in RASMC, p190A is transiently inactivated after 5 min of ANG II stimulation and this inactivation is associated with p190A dephosphorylation. p190A is known to be inhibited by dephosphorylation processes on Tyr1105 by the PTP-PEST or LMW-PTP phosphatases (26, 33). Here we identify that under ANG II stimulation, the SHP2 tyrosine phosphatase dephosphorylates the residue Tyr1105 of p190A thereby inducing its inactivation. Previous studies have already suggested that AT1R signaling could involve SHP2. Indeed SHP-2 appears to have an essential role in Janus kinase JAK2 phosphorylation and initiation of the ANG II-induced JAK/(signal transducers and activators of transcription) STAT cascade leading to smooth muscle cell proliferation (23). More than this, SHP2 can dephosphorylate and inactivate RhoA GEFs (35) in particular the RhoA GEF Vav2 in ANG II-stimulated cells (47). Our data showed that SHP2 activity did not only control p190A phosphorylation in response to ANG II but, by another way, RhoA-ROCK pathway activation (Fig. 5A). Therefore, in addition to p190A inhibition, SHP2 could control the activity of a RhoA GEF such as Vav2 or LARG, recently suggested to participate in ANG II-induced responses (48).

In addition, large literature described how AT1R activates SHP2, in particular through reactive oxygen species generation.
and oxidation (9, 13, 23). On the other hand, AT2R inhibits superoxide production and could thereby inhibit SHP2 (11, 37, 39). That could explain the greater effect observed in the presence of AT2R antagonist and let us also suspect a tonic induction of p190A phosphorylation by AT2R stimulation.

Eventually, we show that activation and phosphorylation of p190A, which is known to be done by c-Src, cAbl, or Agr tyrosine kinases (4, 15, 16), also involves SHP2. Indeed, SHP2 is a tony activator of p190A, not by its catalytic activity, but by maintaining p190A bound to the cAbl-tyrosine kinase. The increased binding of cAbl to the dephosphorylated form of p190A (Fig. 4A) and the additional activation of cAbl in response to AT1R stimulation [Fig. 5B (45)] suggest that these mechanisms are designed to rapidly restore p190A phosphorylation and activity and quickly limit AT1R-induced RhoA/ROCK activation. A previous work has described that cAbl had an inhibitory effect on RhoA pathway because its activity was always inversely related to RhoA activity in neurons (20) and the Arg member of cAbl family has been shown to phosphorylated p190A in brain (17). We demonstrate here that in VSMC, cAbl is bound to p190A and constantly activates it to maintain a low level of RhoA activity. Very recently, ROCK has been shown to phosphorylate the Ser1150 of p190A to attenuate p190A GAP activity and prolong RhoA-ROCK activation in response to high concentration of endothelin-1, growing stronger the importance of p190A to allow an adequate response of VSMC to vasoactive agents (25).

Our work strengthens the central role of SHP2 in ANG II signaling downstream to AT1R. As a scaffold protein, SHP2 is necessary to allow cAbl/p190A association and cAbl-mediated p190A phosphorylation to maintain basal p190A activation and consequently a low RhoA-ROCK activity in RASMC. Additionally SHP2 phosphatase activity is necessary to promote p190A dephosphorylation and inhibition in response to ANG II. This study outlines the crucial role of GAP in the regulation of RhoA activity and more precisely the role of p190A in the response to ANG II.

ACKNOWLEDGMENTS

We thank Dr. Sarah J. Parson for the gift of the plasmids encoding p190A, and Nathalie Vaillant and Cindy Schleder for skillful technical assistance.

GRANTS

Malvyne Rolli-Derkerdiner is supported by the Centre National pour la Recherche Scientifique (CNRS). This work was supported by grants from the Programme National de Recherche sur les Maladies Cardiovasculaires 2007 (project number A07105NS) and from the Institut National de la Sante et de la Recherche Medicale (INSERM).

REFERENCES