PDGF-dependent regulation of regulator of G protein signaling-5 expression and vascular smooth muscle cell functionality

Jagadambika J. Gunaje,1 Arya J. Bahrami,1 Stephen M. Schwartz,1 Guenter Daum,2 and William M. Mahoney, Jr.1

Departments of 1Pathology and Center for Cardiovascular Biology, and 2Surgery, University of Washington, Seattle, Washington

Submitted 26 August 2010; accepted in final form 13 May 2011

Gunaje JJ, Bahrami AJ, Schwartz SM, Daum G, Mahoney WM, Jr. PDGF-dependent regulation of regulator of G protein signaling-5 expression and vascular smooth muscle cell functionality. Am J Physiol Cell Physiol 301: C478–C489, 2011. First published May 18, 2011; doi:10.1152/ajpcell.00348.2010.—Regulator of G protein signaling (RGS) proteins, and notably members of the RGS-R4 subfamily, control vasocontractility by accelerating the inactivation of G protein subunits. To determine which physiological agonists directly control RGS5 expression in vascular smooth muscle cells (VSMCs), we demonstrate RGS5 overexpression inhibits G protein-coupled receptor (GPCR)-mediated hypertrophic responses. These results demonstrate RGS5 overexpression inhibits G protein-coupled receptor (GPCR)-mediated hypertrophic responses. The next objective was to determine which physiological agonists directly control RGS5 expression in VSMCs. GPCR agonists failed to directly regulate RGS5 mRNA expression; however, platelet-derived growth factor (PDGF) acutely represses expression. Downregulation of RGS5 results in the induction of migration and the activation of the GPCR-mediated signaling pathways. This stimulation leads to the activation of mitogen-activated protein kinases directly downstream of receptor stimulation, and ultimately VSMC hypertrophy. These results demonstrate that RGS5 expression is a critical mediator of both VSMC contraction and potentially, arterial remodeling.

regulator of G protein signaling; platelet-derived growth factor; G protein-coupled receptor; vasoactive agonists; cardiovascular signaling

ANGIOTENSIN, endothelin, thrombin, acetylcholine, and catecholamines are major regulators of both smooth muscle contraction and arterial wall mass. All of these agonists transmit their signals through G protein-coupled receptors (GPCRs), a family of seven genes that comprise ~1% of the mammalian genome (11). GPCR-mediated signaling has many implications for vascular disease. The characterization of the receptor-agonist interaction should be and has been an important therapeutic target for both systemic and pulmonary hypertension. An equally important target may be the complex of regulatory molecules that determine the extent and duration of GPCR signaling within the vascular smooth muscle cell (VSMC). Expression of different regulators may determine very different functions for the same GPCR in different cells. One such group of regulatory protein, the regulator of G protein signaling (RGS) proteins, has been implicated in controlling the function of G protein-coupled receptor (GPCR) signaling pathways downstream of activation of G proteins (5, 20, 32, 37, 58, 82, 83). Modulation of GPCR signaling by RGS proteins depends on the function of G protein subunits as activators of GTPase activity for GTP-bound Go large G proteins (9, 25). Because of this activity, members of the RGS-R4 subfamily appear to be critical to cardiovascular function and pathology (5, 62). For example, cardiac-directed overexpression of RGS5 and RGS4 results in the failure to efficiently remodel in response to pressure overload (41, 59, 60). RGS2 has been linked to blood pressure regulation, presumably via modulation of vasoconstrictor activity (31, 38, 56, 66, 70). We have demonstrated RGS5 is preferentially expressed in aortic SMCs, relative to venous SMCs (1, 2). Developmental studies and studies of tumor angiogenesis suggest RGS5 may be critical to vascular stability in newly formed vascular beds (7, 34, 51). Finally, we (55) and others (18, 22, 30) determined that RGS5 is also linked to blood pressure regulation.

The present study is aimed at determining which physiological agonists control RGS5 expression in vascular SMCs. Very little is known about regulation of expression in this gene family other than the observation that ANG II upregulates RGS2 expression in vitro, both at the transcript and protein level (43, 61), whereas sphingosine-1-phosphate (S1P) upregulates RGS2 and RGS16 expression (36). In contrast, we found that RGS5 expression is not directly regulated by any of the seven candidate GPCR agonists assayed following either 2, 6, or 24 h of stimulation. We previously demonstrated RGS5 expression is downregulated in response to aortic constriction (79). Perhaps explaining this in vivo response, RGS5 expression is downregulated in response to platelet-derived growth factor (PDGF) treatment. This results in increased migration and hypertrophic signaling in VSMCs. These results suggest that the role of PDGF in the vascular response to injury may be mediated by cross-talk between the growth factor and GPCR signaling pathways.

MATERIALS AND METHODS

Cell Culture

All cells lines were derived from the rat aorta. The RGS5− VSMC line is described in Wang et al. (79) and was cultured in DMEM (GIBCO) and supplemented with 10% FBS (Hyclone) and penicillin-streptomycin. The RGS5+ VSMC line was kindly provided by Dr. Gary Owens [University of Virginia (68)] and was cultured in DMEM/F12 (GIBCO), supplemented with 10% FBS (Hyclone) and penicillin-streptomycin. All cells are grown at 37°C and 5% CO2.

Address for reprint requests and other correspondence: W. M. Mahoney, Jr., Center for Cardiovascular Biology Univ. of Washington, 815 Mercer St., RM 435, Seattle, WA 98109 (e-mail: wmahoney@u.washington.edu).
Importantly, RGS5+ VSMCs express members of the RGS-R4 subfamily at endogenous levels (see Fig. 2).

**Viral Overexpression of RGS5**

The construction and production of RGS5 retrovirus are described in Wang et al. (79).

**siRNA Knockdown of RGS5 Expression**

RGS5 was knocked down in RGS5+ VSMCs using a specific small interfering RNA (siRNA) from Invitrogen (5'-AAUUCUCAGCGAACCAGAACCUC-3'). VSMCs were transfected by electroporation with the human AoSMC nucleofector kit (Amaxa Biosystems) following manufacturers specifications. Briefly, 6 × 10^6 cells were transfected with either RGS5-specific siRNA (40 nM) or nonspecific siRNA (40 nM; Invitrogen) and grown in complete growth media. After 24 h, the cells were changed to serum-free media and starved for 24 h. Cells were subsequently treated with the following agonists for 24 h: ANG II (100 nM; Sigma), endothelin-1 (ET-1; 100 nM; Sigma), phenylephrine (PE; 10 μM; Sigma), isoproterenol (Iso; 10 μM; Sigma), serotonin (5-HT; 5 μM; Sigma), norepinephrine (NE; 10 μM; Sigma), sphingosine-1-phosphate (S1P; 1 μM; Cayman), PDGF (10 ng/ml; R&D systems), vascular endothelial growth factor (VEGF; 10 ng/ml; R&D systems), and epidermal growth factor (EGF; 10 ng/ml; R&D systems).

**Quantitative Real-Time RT-PCR**

RNA was isolated from VSMCs with or without agonist treatment using the RNAEasy RNA isolation mini kit (Qiagen). After determination of RNA quantity and quality, cDNA was prepared by reverse transcription (Reverse Transcription cDNA Synthesis kit; Applied Biosystems), using random hexamer primers. Real-time PCR was performed by mixing 20 ng cDNA with 20× primer-probe mixes and 2× Taqman PCR Master Mix (Applied Biosystems), and the quantity of each product was determined on the 7900HT Real Time PCR machine (Applied Biosystems). Thermal cycling for PCR was as follows: 2 min at 50°C, 10 min at 95°C, followed by 40 cycles of 15 s at 95°C for denaturation and 1 min at 60°C for annealing and extension. The amount of each target molecule mRNA was calculated using a comparative Ct method (2^(-ΔΔCt); Applied Biosystems, Relative Quantitation Of Gene Expression, ABI PRISM 7700 Sequence Detection System: User Bulletin no. 2), after normalized to glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (48). The rat genes assayed were the following: RGS2 (Rn00584932_m1; Applied Biosystems), RGS4 (Rn00568067_m1; Applied Biosystems), RGS5 (Rn00571047_m1; Applied Biosystems), and GAPDH (Rn99999916_s1; Applied Biosystems).

**Immunoblot**

Protein cell lysates were prepared from VSMCs following 0, 2, 5, 10, 30, and 60 min of agonist treatment. Briefly, cells were washed and scraped into 1× PBS. Whole cell extracts were prepared by resuspending the cell pellet in lysis buffer [50 mM Tris·HCl (pH 8.0), 1 mM EDTA, and protease inhibitor resuspending the cell pellet in lysis buffer [50 mM Tris·HCl (pH 8.0), 1 mM EDTA, and protease inhibitor](see Fig. 1). Using [3H]leucine incorporation as a measure of hypertrophic protein production, we demonstrate PE, ET-1, and ANG II-induced hypertrophy is inhibited by approximately 25% on average (25%, 18%, and 36%, respectively). Therefore, in VSMCs, RGS5 interacts with Goq and Goq and effectively inhibits GPCR-mediated hypertrophy.

Characterization of an In Vitro Model System: Expression of RGS5 in a Cultured VSMC Line

**Rat aortic VSMCs express RGS-R4 subfamily members.** Because of the concern with the physiological relevance of overexpression of a regulator of signal transduction, we explored the physiological role of RGS-R4 proteins in an in vitro rat aortic cell line that expresses RGSS and other members of
Regulator of G protein signaling 5 (RGS5) overexpression inhibits hypertrophy in vascular smooth muscle cells (VSMCs). Rat aortic VSMCs were infected with retrovirus overexpressing either RGS5 or green fluorescent protein (GFP) (control). After stimulation with the G protein-coupled receptor (GPCR) agonists [10 μM phenylephrine (PE), 100 nM endothelin-1 (ET-1), 100 nM ANG II] for 24 h and pulsed with 1 μCi [3H]leucine for 5 h, the amount of [3H]leucine incorporated into new protein was quantitated to assess hypertrophic growth. Each GPCR agonist assayed stimulated hypertrophic growth of VSMCs. When RGS5 was overexpressed, but not when GFP was overexpressed, hypertrophic growth was inhibited. Error bars are means ± SE; n = 3.

Relative quantitative real-time RT-PCR analysis demonstrates RGS5 and RGS4 are approximately equally expressed, whereas RGS2 is expressed at lower levels (Fig. 2A). This closely relates to additional analyses from our laboratory demonstrating the differential expression pattern for individual members of the RGS-R4 subfamily in the rat and the mouse aorta in vivo: RGS5 > RGS4 > RGS2 > RGS16 (data not shown). While an absence of sensitive antibodies makes protein quantification of the RGS-R4 subfamily members difficult, we propose the modulation of RGS mRNA expression and the subsequent measurement of GPCR signaling activity is the best approach to study the functional effect of RGS-R4 expression changes.

Knockdown of RGS5 by siRNA is specific to RGS5 relative to other closely related RGS-R4 subfamily members. RGS proteins have highly conserved structures and functions throughout evolution. This is of particular importance when comparing members of the RGS-R4 subfamily, since this family is structurally described as having short NH2- and COOH-terminal domains and the catalytic RGS domain, which comprises approximately two-thirds of the protein (25, 69). Therefore, when specifically targeting one member of the family with a siRNA, it is necessary to determine whether the expression of additional members is accidently modified. As demonstrated in Fig. 2B, relative to RGS2, RGS4, and RGS16, we have specifically targeted RGS5 by siRNA knockdown. Furthermore, we analyzed RGS5 expression 24 h following knockdown with two additional siRNAs (see supplemental Figs. S1 and S2 online at the AJP-Cell Physiol website) and confirmed that multiple independent siRNAs have equivalent effects upon RGS5 expression. Therefore, in subsequent studies, we are confident we have knocked down RGS5, and we can attribute the functional response in these cells to RGS5 expression (or loss thereof).

Regulator of G protein signaling 5 (RGS5) expression regulates ANG II-stimulated signaling pathways. ANG II has been shown to activate phosphorylation of mitogen-activated protein kinases (MAPKs) (74, 84). Importantly, phosphorylation of mitogen-activated protein kinases (MAPKs) has been implicated in the contractile, mitogenic, and trophic responses of arterial VSMCs (16, 42, 44, 47, 67, 86). Therefore, to determine whether RGS5 expression regulates ANG II-stimulated hypertrophy, we focused on the effects of RGS5 knockdown on this signaling pathway. ANG II has been shown to activate multiple signaling pathways (74, 84). Knockdown of RGS5 activates endogenous GPCR-mediated signaling in VSMCs. RGS proteins, as GTPase activating proteins (GAPs) (24, 27, 62, 87), function to inhibit GPCR signaling through GαGTP-dependent pathways. Since overexpression of RGS5 affected the ANG II-dependent hypertrophic response, we focused on the effects of RGS5 knockdown on this signaling pathway. ANG II has been shown to activate multiple signaling pathways (74, 84). Knockdown of RGS5 activates endogenous GPCR-mediated signaling in VSMCs.
II-dependent signaling, the effect of specific knock down of RGS5 upon MAPK stimulation was analyzed in vitro.

Figure 2C demonstrates that targeted knockdown of RGS5 results in the potentiation of ANG II-mediated activation of multiple downstream MAPKs. Twenty-four hours after siRNA treatment, VSMCs were stimulated with 100 nM ANG II. Relative to the VSMCs treated with nonspecific siRNA, phosphorylated ERK (p42/p44) was markedly activated in the VSMCs treated with RGS5 siRNA. A similar response was observed for the two additional RGS5-specific siRNAs assayed (see online supplemental Fig. S2). This response is quantified in Fig. 2D. This induction occurred within 2 min after ANG II treatment and diminished after 10 min of ANG II treatment. Phosphorylation of two additional kinases, Akt and JNK, was...
simply activated within 2 min of stimulation, although the phosphorylation was not as sustained as observed for pERK. Similarly, RGS5-dependent responses were observed for additional GPCR agonists studied (ET-1, PE, Iso, S1P, NE, 5-HT; see supplemental Fig. S3, A–F). In summary, RGS5 expression clearly controls the GPCR-mediated rapid and transient increase in kinase phosphorylation in VSMCs.

RGS5 inhibition activates the hypertrophic response in VSMCs. To confirm the activation of functional cascades downstream of ANG II stimulation, the analysis of hypertrophic incorporation of [3H]leucine was analyzed in the presence and absence of RGS5-specific siRNA. As expected, knockdown of RGS5 resulted in an approximately fivefold increase in hypertrophic protein production (Fig. 2E). Interestingly, knockdown of RGS5 expression does not sensitize VSMCs to the ANG II-mediated hypertrophic response. Specifically, an induction of hypertrophy is only observed when VSMCs are stimulated with 100 nM ANG II but not at lesser ANG II concentrations (either 10 or 50 nM). As above, similar RGS5-dependent hypertrophic responses were observed for additional GPCR agonists studied (ET-1, PE, Iso, S1P, NE, 5-HT; see Supplemental Fig. S3G). Taken together, these data indicate that in VSMCs, RGS5 expression controls both the transient signaling events (MAPK activation is largely complete 10 min after ANG II stimulation; Fig. 2, C and D) and the more long-term physiological effects in response to GPCR stimulation (hypertrophic growth is assayed 29 h following ANG II stimulation; Fig. 2E), and specifically, the signaling pathways stimulated by ANG II treatment.

Agnost-Dependent Regulation of RGS5 Expression

GPCR agonists do not directly activate RGS5 expression in VSMCs. Expression of some members of the RGS-R4 subfamily is directly controlled by GPCR agonist stimulation. For example, RGS2 expression is upregulated in response to ANG II treatment both in cultured VSMCs (43) and in the adrenocortical carcinoma cell line (61). To determine whether RGS5 expression is similarly induced in response to GPCR stimulation, VSMCs were treated with ANG II, ET-1, PE, Iso, 5-HT, NE, and S1P, and the expression of RGS5 was determined by quantitative real-time RT-PCR. In contrast to the described induction of RGS2 in response to ANG II stimulation, none of the GPCR agonists studied significantly affected RGS5 expression levels following 2, 6, or 24 h of stimulation (Fig. 3A). As demonstrated in Fig. 2, C and D, when RGS5 is knocked down, ANG II-mediated phosphorylation of ERK, Akt, and JNK occurs rapidly. Therefore, to investigate whether short-term GPCR stimulation had any affect upon RGS5 expression, we assayed a few candidate GPCR agonists (ANG II, PE, ET-1) for changes in RGS5 mRNA levels. As expected from the more extended stimulation assays, acute stimulation by these three candidate agonists had no significant effect upon RGS5 expression (Fig. 3B). Taken together, this indicates an alternative mechanism(s) is controlling expression of RGS5, and potentially additional candidate RGS-R4 subfamily members, in cultured VSMCs.

PDGF-BB stimulation directly represses RGS5 expression in VSMCs. We are interested in the possibility that PDGF might regulate RGS5 expression because RGS5 and the PDGF-Rβ are expressed in pericytes, which function to stabilize neovascularure (7, 12, 13, 21, 53). The effect of PDGF upon a GPCR-mediated pathway is not unexpected because there is growing evidence of cross-talk between GPCRs and receptor tyrosine kinases (RTKs) (40, 54, 78, 81). Treatment of VSMCs with PDGF-BB resulted in the immediate and sustained downregulation of RGS5 expression (Fig. 3C). As shown, RGS5 expression is downregulated by 25%, 50%, and 80% following 2, 6, and 24 h of PDGF stimulation, respectively. Importantly, this effect is specific to PDGF, whereas additional RTK stimulants (VEGF and EGF) failed to alter RGS5 expression.

PDGF isoforms have different effects on RGS-R4 subfamily member expression. Figure 3C clearly demonstrates that PDGF-BB treatment downregulates RGS5 expression. Unfortunately, PDGF-BB binds all conformations of the PDGF receptor in vitro: the PDGFα homodimer, the PDGFβ homodimer, and the PDGFαβ heterodimer (3, 71). Therefore, to determine which receptor is responsible for the downregulation of RGS5, VSMCs were treated with each PDGF isoform (PDGF-AA, -BB, -CC, and -DD). In addition to RGS5 expression, the expression of both RGS4 and RGS2 was also determined by quantitative real-time RT-PCR in response to each of the PDGF isoforms.

In VSMCs, the PDGF-AA and PDGF-CC isoforms did not significantly affect RGS2, RGS4, or RGS5 expression following stimulation for 2, 6, and 24 h (Fig. 3D). Conversely, the PDGF-BB and PDGF-DD isoforms regulated expression of these RGS-R4 subfamily members. RGS5 and RGS4 were downregulated by both PDGF-BB and PDGF-DD throughout the stimulation time course. Interestingly, RGS2 was acutely induced by PDGF-BB and PDGF-DD stimulation; however, expression decreases in a similar pattern to both RGS5 and RGS4 following 6 and 24 h of stimulation. As with the published activation of RGS2 by ANG II (43, 61), our results provide another example of different regulatory mechanisms controlling the expression of these different RGS-R4 subfamily members. Finally, since an equivalent effect on RGS5 and RGS4 expression is observed following PDGF-BB and PDGF-DD treatment, and PDGF-BB has been shown to only
RGS5 was knocked down. Treatment with VEGF failed to induce VSMC migration when quantitated (Fig. 4). To confirm that this effect was dependent on PDGF, cells were treated with VEGF and the number of migrated cells was quantitated (Fig. 4A). As shown in Fig. 4, treatment with PDGF-BB for 24 h, followed by a short time course of ANG II stimulation, resulted in an equivalent, yet transient, increase in phosphorylated MAPK (Fig. 5A and Supplemental Fig. S5). Conversely, when cells were not treated with PDGF-BB for 24 h, ANG II-dependent pERK stimulation is not observed in these RGS5−/− VSMCs. The combined effect of PDGF-BB and ANG II on pERK stimulation is quantitated in Fig. 5B. This indicates the GPCR-mediated activation of pERK is dependent on first knocking down RGS5 expression by PDGF-BB treatment. Importantly, not only are the signaling pathways downstream of ANG II stimulated, but the hypertrophic response in VSMCs is also activated by the combination of PDGF-BB and ANG II treatment. Similar to the effect in VSMCs treated with ANG II and RGS5-specific siRNA, VSMCs treated with PDGF-BB for 24 h followed by ANG II treatment exhibited a sixfold increase in hypertrophic protein production (Fig. 5C). Also, as observed following specific knockdown of RGS5 by siRNA (Fig. 2F), PDGF-mediated knockdown of RGS5 does not sensitize VSMCs to the ANG II-mediated hypertrophic response. As predicted by the siRNA experiment, hypertrophic growth is only observed when VSMCs are stimulated with 100 nM ANG II but not at the lesser ANG II concentrations. To confirm RGS5 expression remained inhibited following PDGF-BB treatment, we assayed RGS5 mRNA expression at each time point of potential agonist addition during the hypertrophy assay. As shown in Supplemental Fig. S4, once RGS5 expression is inhibited, it remains repressed throughout the entire 53-h experimental time course. Combined, these data provide the first evidence of RTK activation directly regulating the expression of a mediator of Gα signaling, RGS5, leading to increased cell migration and enhanced hypertrophic signaling.

DISCUSSION

Beyond its role in controlling blood pressure (18, 22, 30, 55), RGS5 has been implicated in the control of vessel branching in normal development (7, 53), as well as in cancer (34, 51). The observations presented here suggest a mechanism by which the PDGF signaling cascade directly regulates GPCR-mediated signaling via RGS5 downregulation, thereby linking vascular remodeling to the intricate control of Gα signaling and the downstream physiological responses (i.e., VSMC migration and hypertrophy).

As expected, we found that RGS5 overexpression inhibits GPCR-mediated hypertrophy in VSMCs (Fig. 1). This might lead one to expect a feedback regulation of RGS5 expression, as has been reported for ANG II-mediated induction of RGS2 in both VSMCs (43) and adrenocortical cells (61). However, we found that expression of RGS5 is neither stimulated nor repressed in response to stimulation with GPCR agonists (Fig. 3, A and B). In contrast, when VSMCs are treated with PDGF, RGS5 expression is inhibited (Fig. 3, C and D). When RGS5 is...
inhibited, either by siRNA (Fig. 2B) or pharmacologically (Fig. 3, C and D), VSMCs become more migratory (Fig. 4) and GPCR-mediated signaling is induced (Figs. 2 and 5). It is interesting to note that the hypertrophic response is stimulated when RGS5 is knocked down, even in the absence of exogenous GPCR agonists (Fig. 2E and Supplemental Fig. S3G). This implies that RGS5 functions to inhibit GPCR-mediated signaling by endogenous agonists present in the culture media. Therefore, by simply knocking out RGS5, signaling, and ultimately hypertrophic growth, is induced. However, when cells are stimulated by additional GPCR agonists, hypertrophic growth is further stimulated in the absence of RGS5. Therefore, RGS5 functions to maintain the “steady-state” of the SMC.

While RGS5 identifies the pericyte and its expression is downregulated in PDGF-BB and PDGFRβ knockout mice, RGS5-null animals do not have a deficiency in pericyte coverage (12, 13, 35, 55). Pericytes are SMC-like cells that function to stabilize newly formed capillaries during angiogenesis, including tumor angiogenesis and development (4, 76). The pericyte is believed to be derived by PDGF-mediated migration of adventitial mesenchymal cells along the axis of newly formed endothelial branches. The process is dependent on endothelial cell-derived PDGF-BB and PDGFRβ (6, 10, 35, 45). PDGF-BB and PDGFRβ knockout mice are characterized by “leaky” and unstable vessels because of the lack of pericytes. Our data suggest that at this stage of pericyte formation, RGS5 expression would be downregulated. RGS5 expression would be upregulated once neovessels are fully encoated by pericytes and are therefore functionally capable of distributing blood flow, as suggested by Mitchell et al. (53). One possible function of RGS5 is the stabilization or organization of the newly formed vessels, perhaps accounting for the observation that RGS5 controls blood flow through experimental tumors in RGS5 knockout mice (34) and the observation of RGS5 as a tumor progression marker (7, 17, 19, 51).

Fig. 5. PDGF-BB stimulates RGS5 knockdown, induces ANG II-mediated ERK phosphorylation, and induces ANG II-mediated hypertrophy. A: VSMCs were stimulated with ANG II (100 nM) for the indicated times, following 24 h treatment of PDGF-BB (10 ng/ml; left) or without PDGF-BB treatment (right). Whole cell extracts were prepared and immunoblotted for the expression of phosphorylated ERK. β-Actin is shown as a control for equivalent protein loading. Note: for comparison, the p ERK blot is overexposed relative to the + nonspecific siRNA blot (Fig. 2E). B: quantitation of immunoblots (n = 4) demonstrating PDGF-BB treatment stimulates ANG II-mediated phosphorylation of ERK. C: PDGF-BB induces ANG II-stimulated hypertrophy. VSMCs were treated with or without PDGF-BB for 24 h, stimulated with ANG II (0, 10, 50, and 100 nM) for 24 h, and pulsed with 1 μCi [3H]leucine for 5 h, and the amount of [3H]leucine incorporated into new protein was quantitated to assess hypertrophic growth. Error bars are means ± SE; n = 3.
This hypothesis is strengthened by our observation that RGS5 also opposes PDGF function. This may open up a new and unsuspected variation on the idea of crosstalk between GPCRs and RTKs (14, 52, 54). Usually this has been thought of in terms of activation of the growth factor receptors following GPCR stimulation (23, 40, 75). Recently, Wang et al. (78) demonstrated signaling through the angiotensin type I receptor (AT1R) activates the PDGFRβ-dependent signaling cascade. However, all of these studies imply a one-way activation of RTK signaling cascades following GPCR stimulation. This mechanism has been referred to as the triple-membrane-passing-signaling model (77, 81). Our data showing PDGFRβ stimulation functions to downregulate RGS5 expression establishes a link between the RTK and GPCR signaling cascade, independent of the GPCR-to-RTK connection established previously.

Taken together, our results have led us to propose the following model (Fig. 6). In the quiescent arterial wall, differentiated VSMCs express RGS5, resulting in the inhibition of GPCR signaling and cells do not migrate or undergo hypertrophy, even in the presence of circulating/local GPCR agonists (Fig. 6A). However, upon vascular injury, the local concentration of PDGF-BB increases, through release from platelets, activated endothelial cells, and invading macrophages (50, 64, 72). As a result, RGS5 expression is inhibited.
and VSMCs become more migratory and proliferative. In addition, the inhibition of RGS5 expression enables signaling through the relevant GPCRs. The specific inhibition of RGS5 results in the phosphorylation-mediated activation of MAPKs downstream of Gαq, and ultimately, VSMC hypertrophy/remodeling (Fig. 6B). Once the injury response is complete, the platelets and macrophage dissipate and the local concentration of PDGF-BB is diminished. As a result, RGS5 expression returns to endogenous levels, and GPCR-mediated signaling is consequently inhibited.

These data correlate with our previously described expression pattern for RGS5 in vivo, in the remodeling response to thoracic aortic banding in the rat (79). Upon vascular injury, the expression of PDGF is induced, along with the expression of Kruppel-like factor 4 (KLF4), which results in a downregulation of genes expressed in differentiated VSMCs, including smooth muscle myosin heavy chain and SM22α/transgelin (26, 46, 85). Acutely, injured arteries also go into spasm, perhaps due to the combined contractile effect of PDGF and the loss of expression of RGS5 (8). In addition to stimulating contraction, PDGF also induces VSMCs to dedifferentiate and become more proliferative and migratory (39), resulting in the classic vascular response to injury.

The data presented here provide a mechanism by which the combined effects of vasoactive GPCR stimulants and the local concentration of PDGF-BB account for the classic vascular response to injury. Furthermore, our results establish a central role for RGS5, and potentially additional members of the RGS-R4 subfamily, in cardiovascular remodeling.

ACKNOWLEDGEMENTS

We thank Dr. Mark Majesky (Seattle Children’s Research Institute) for useful discussions and for critically reading this manuscript.

GRANTS

This work was supported by the National Heart, Lung, and Blood Institute Grants HL-007312 and HL-087513.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

REFERENCES


prolifering in two models of hypertension identifies Cav-1, Rgs2 and Rgs5 as antihypertensive targets. BMC Genomics 8: 408, 2007.


