Regulation of the Platelet-derived Growth Factor Receptor-β by G Protein-coupled Receptor Kinase-5 in Vascular Smooth Muscle Cells Involves the Phosphatase Shp2*

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Smooth muscle cell (SMC) proliferation and migration are substantially controlled by the platelet-derived growth factor receptor-β (PDGFRβ), which can be regulated by the Ser/Thr kinase G protein-coupled receptor kinase-2 (GRK2). In mouse aortic SMCs, however, we found that prolonged PDGFRβ activation engendered down-regulation of GRK5, but not GRK2; moreover, GRK5 and PDGFRβ were coordinately up-regulated in SMCs from athero-sclerotic arteries. With SMCs from GRK5 knock-out and cognate wild type mice (five of each), we found that physiologic expression of GRK5 increased PDGF-promoted PDGFRβ seryl phosphorylation by 3-fold and reduced PDGFRβ-promoted phosphoinositide hydrolysis, thymidine incorporation, and overall PDGFRβ tyrosyl phosphorylation by ~35%. Physiologic SMC GRK5 activity also increased PDGFRβ association with the phosphatase Shp2 (8-fold), enhanced phosphorylation of PDGFRβ Tyr1021 (the docking site for Shp2), and reduced phosphorylation of PDGFRβ Tyr1022. Consistent with having increased PDGFRβ-associated Shp2 activity, GRK5-expressing SMCs demonstrated greater PDGF-induced Src activation than GRK5-null cells. GRK5-mediated desensitization of PDGFRβ inositol phosphate signaling was diminished by Shp2 knock-down or impairment of PDGFRβ/Shp2 association. In contrast to GRK5, physiologic GRK2 activity did not alter PDGFRβ/Shp2 association. Finally, purified GRK5 effected agonist-dependent seryl phosphorylation of partially purified PDGFRβs. We conclude that GRK5 mediates the preponderance of PDGFRβ-promoted seryl phosphorylation of the PDGFRβ in SMCs, and, through mechanisms involving Shp2, desensitizes PDGFRβ inositol phosphate signaling and enhances PDGFRβ-triggered Src activation.

The pathogenesis of atherosclerosis (1) and neointimal hyperplasia after vascular injury (2) fundamentally involves the platelet-derived growth factor receptor-β (PDGFRβ) expressed on smooth muscle cells (SMCs) (3). As a receptor tyrosine kinase, the PDGFRβ autophosphorylates on tyrosyl residues upon binding PDGF. Subsequently, the PDGFRβ activates intracellular signaling cascades by tyrosine-phosphorylating and/or scaffolding multiple proteins critical for cellular proliferation and migration (4). Until they are destroyed in the lysosome, activated PDGFRβs appear to continue signaling (5). Thus, regulation of PDGFRβ signaling prior to receptor degradation attains considerable significance.

Along with PDGFRβ tyrosyl dephosphorylation (4), an important mechanism for bridging PDGFRβ signaling involves PDGFRβ phosphorylation on seryl residues (6, 7). We have recently demonstrated that the PDGFRβ undergoes agonist-dependent seryl phosphorylation by G protein-coupled receptor kinase-2 (GRK2), a ubiquitously expressed Ser/Thr kinase (7, 8). GRK2-mediated phosphorylation of the PDGFRβ results in diminished PDGFRβ signaling, or desensitization, through mechanisms related to reduced PDGFRβ/Na+/H+ exchanger regulatory factor association (8), reduced PDGFRβ tyrosyl phosphorylation (7–9), and PDGFRβ hyperubiquitination (7). This PDGFRβ desensitization manifests as diminished second messenger signaling (9) and phosphoinositide 3-kinase activation (10) in the short term, and manifests as diminished SMC migration (10), thymidine incorporation (9, 10), and proliferation (9) in the medium to long term.

GRK2 belongs to a seven-member family of Ser/Thr kinases (11, 12), each with a central catalytic domain flanked by amino-and carboxyl-terminal domains that serve membrane-localizing, protein association, and other regulatory functions (11). Allosterically activated by agonist-occupied heptahelical (sev-en-membrane-spanning) receptors, GRKs characteristically phosphorylate these activated receptors and thereby initiate desensitization that is “receptor-specific” (i.e. that affects only the receptor whose activation prompted GRK activity). Only GRKs 2, 5, and 6 are widely expressed at substantial levels in mammalian tissues. With only 58.6% similarity to GRK2 (13), GRK5 has demonstrated receptor substrate specificity both

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5 The abbreviations used are: PDGFRβ, PDGF receptor-β; PDGF, platelet-de- rivied growth factor; SMC, vascular smooth muscle cell; GRK, heterotrimeric G protein-coupled receptor kinase; IP, immunoprecipitation or immunoprecipitate; IB, immunoblot(s), immunoblotting; KO, knockout; EGF, epidermal growth factor; PLC, phospholipase C; siRNA, small interfering RNA; MEF, mouse embryo fibroblast; WT, wild type; ELISA, enzyme-linked immunosorbent assay; PBS, phosphate-buffered saline; ERK, extracellular signal-regulated kinase.
overlapping with (13) and distinct from (14, 15) GRK2. GRK subtype-specific phosphorylation sites (16) have been shown to result in distinct downstream molecular consequences for receptors phosphorylated by both GRK2 and GRK5 (17–20). In light of the role of GRK2 in regulating PDGFRβ function in fibroblasts, we initiated this investigation to determine whether the PDGFRβ can be regulated in SMCs by GRK5 and whether GRK5 and GRK2 employ similar or distinct mechanisms for PDGFRβ regulation.

MATERIALS AND METHODS

Recombinant Adenoviruses and Plasmids—The cDNA encoding bovine GRK5 was inserted into the plasmid pSKAC, and adenoviruses were produced in 293 cells, purified on CsCl gradients, and titered as we described previously (9). Plasmids encoding the N-terminal FLAG™-tagged human PDGFRβ (FPDGFRβ) (7), bovine GRK2 (21), and bovine GRK5 (21) have been described previously. The Y1009F mutant FPDGFRβ was created from its WT congener by cassette PCR, using the following primers: 5′-cgcggccgatctcgccgacccgaatg-3′ (underscore denotes the HindIII site) and 5′-cgcggccgatccctctgccgagcccaatg-3′ (underscore denotes the NotI site). PCR fidelity was verified by dye-deoxy sequencing.

Atherosclerosis Studies in Mice—All animal care conformed to Ref. 59. C57Bl/6J mice without (wild type, WT) or with targeted deletion of the apolipoprotein E gene (apo−/−; Jackson stock number 002052) were purchased from Jackson Laboratories and fed normal mouse chow. Nine-month-old mice were sacrificed simultaneously for SMC production, and all comparisons were made among littermate-derived SMCs. SMCs were used only through passage 7. Infections of SMCs with recombinant adenoviruses were performed at equivalent multiplicities of infection (from 50 to 100), and cells were assayed 48–72 h after infection, as we described previously (9). In GRK5 “add-back” experiments with grk5−/− SMCs, assays were performed 6 h after adenovirus infection (when GRK5 expression levels were equivalent to those obtaining in WT SMCs).

To assay cell surface PDGFRβ expression levels in transfected HEK cells, we used cell surface immunofluorescence and flow cytometry, as we described previously (7). Cell surface PDGFRβ expression levels of all cell lines were within 30% of control cell values; cell lines outside of this range were not used to generate data. Compared with HEK cells co-transfected with empty vector plasmid, HEK cells overexpressing either GRK2 or GRK5 demonstrated equivalent (−35%) reductions in PDGF-promoted phosphoinositide hydrolysis; GRK expression was 20–40-fold over endogenous levels (data not shown).

SMC Migration and [3H]Thymidine Incorporation—SMC migration was assayed with a protocol modified from one we reported previously (10). SMCs were serum-deprived for 72 h after adenovirus infection, trypsinized, transiently (<5 min) treated with 8% fetal bovine serum (to neutralize the trypsin), and washed with low mitogen medium. Next, SMCs were plated onto Transwell™ membranes (8-μm pores; Costar) in 24-well dishes and allowed to attach for 4 h. PDGF-BB or vehicle was then delivered outside of the Transwell™ membranes, and SMCs were allowed to migrate for 16 h before fixation with methanol. (Pilot studies demonstrated that originally quiescent SMCs do not divide during this time period with PDGF stimulation [data not shown].) SMC nuclei were stained with Hoechst 33342, imaged by fluorescence microscopy, and counted electronically, as we reported previously (10). SMC [3H]thymidine incorporation was assessed during the final 4 h of a 24-h agonist stimulation, as we reported previously (9). Parallel aliquots of SMCs were subjected to lysis and IB and demonstrated that control and GRK5-overexpressing SMCs expressed equivalent levels of the PDGFRβ (data not shown).
**PDGFRβ Regulation by GRK5 in SMCs**

**Immunoblotting and Immunoprecipitations**—These assays used antibodies and procedures described previously (7–9). Immunoprecipitation (IP) of endogenous PDGFRβs used either rabbit or goat anti-PDGFRβ IgG (Santa Cruz Biotechnology), whereas IP of transfected (N-terminal epitope-tagged) PDGFRβ constructs (WT and Y1009F) used anti-FLAG M2-agarose (Sigma). IB employed goat anti-PDGFRβ phospho-Tyr1009, -Tyr1021, and -Tyr740 as well as murine or rabbit anti-Shp2 (Src homology 2 domain-containing protein-tyrosine phosphatase-2) (all from Santa Cruz Biotechnology), anti-c-Src and anti-c-Src phospho-Tyr416 (anti-activated c-Src) (Calbiochem), and anti-PDGFRβ phospho-Tyr729 (GeneTex, Inc.). Phosphorylated PDGFRβ and co-immunoprecipitated band densities were normalized to cognate PDGFRβ band densities, as described previously (8). PDGFRβ tyrosyl and seryl phosphorylation were equivalent after 5 or 10 min of PDGF stimulation (data not shown). The efficiency of PDGFRβ IP was greater than 95%, as assessed by PDGFRβ IB performed simultaneously on post-IP supernatants and serially diluted pre-IP SMC lysate aliquots (data not shown).

**Phosphoinositide Hydrolysis**—SMCs were metabolically labeled with [3H]inositol and stimulated as indicated to provoke phosphoinositide hydrolysis as we described previously (9, 24), except that we used HEPES-buffered saline (pH 7.4) (20 mM HEPES, 20 mM LiCl, 110 mM NaCl, 5 mM KCl, 1 mM MgCl₂, 1 mM CaCl₂) during SMC stimulation and LiCl preincubation. Fluooroaluminate (Al(F₃⁻)³⁻) was used as described (24), and we calculated the percent conversion of [3H]inositol into inositol phosphates as we described previously (24). In assays with mouse SMCs, 2 μM PDGF-AA evoked only 14 ± 2% as much phosphoinositide hydrolysis as 2 μM PDGF-BB (n = 4 SMC lines); thus, 83 ± 2% of our PDGF-BB-dependent mouse SMC phosphoinositide hydrolysis resulted from SMC PDGFRβ activity (4). We therefore elected not to down-regulate the SMC PDGFRα before stimulating the SMC PDGFRβ in phosphoinositide hydrolysis experiments.

**SMC Proliferation**—SMC proliferation was quantitated by enzyme-linked immunosorbent assay (ELISA) for the nuclear enzyme, as described previously (8). PDGFRβ calcified the percent conversion of [3H]inositol into inositol phosphates as we described previously (24). In assays with mouse SMCs, 2 μM PDGF-AA evoked only 14 ± 2% as much phosphoinositide hydrolysis as 2 μM PDGF-BB (n = 4 SMC lines); thus, 83 ± 2% of our PDGF-BB-dependent mouse SMC phosphoinositide hydrolysis resulted from SMC PDGFRβ activity (4). We therefore elected not to down-regulate the SMC PDGFRα before stimulating the SMC PDGFRβ in phosphoinositide hydrolysis experiments.

**RNA Interference**—Small interfering RNAs were chemically synthesized for Shp2 (5'-cccccaagauaugcaguc-3', residues 1080–1100 of the murine sequence) (26), GRK2 (5'-aagaaauagagaugggag-3', residues 172–291 of the murine sequence) (19), and GRK5 (5'-cccucgaaagacctc-3', residues 408–427 of the murine sequence) (19) (Dharmacon, Inc.). Mouse SMCs were grown in 12-well dishes and transfected with mRNA-specific or negative control siRNA (5'-auuucugcagugacagu-3'; Dharmacon) at a concentration of 100 nM, using Lipofectamine 2000 (Invitrogen) according to the manufacturer’s instructions, except for the following: immediately before transfection, SMC growth medium was changed to serum-free OPTI-MEM I; after adding the solution of siRNA and Lipofectamine 2000 to the SMCs, we incubated SMCs at 37 °C in a CO₂ incubator for only 4 h, after which time we added 1 volume of 2x growth medium (without antibiotics) and continued incubation for an additional 48 h. Growth medium was then replaced with serum-free medium (Dulbecco’s modified Eagle’s medium, 20 mM HEPES, pH 7.4, 0.1% (w/v) bovine serum albumin), containing (phosphoinositide hydrolysis) or lacking (IB) 1 μCi of [3H]inositol/ml for a further 20 h. Assays (phosphoinositide hydrolysis and IB) were performed 72 h after siRNA transfection. Flow cytometry of SMCs transfected with fluorescein isothiocyanate-labeled and unlabeled siRNA (Dharmacon) demonstrated the efficiency of transfection under these conditions to be 90–95% (data not shown).

**Statistical Analyses**—Results from multiple experiments were averaged for independent groups but analyzed pairwise, within experiments, by repeated measures analysis of variance and Tukey’s post hoc test for multiple comparisons (Prism 2™ Software, GraphPad, Inc.). Data are presented in the text as mean ± S.D. and in the figures as mean ± S.E.
RESULTS

In cell systems in which GRK2 mediates most of the PDGF-induced seryl phosphorylation of the PDGFRβ, persistent PDGFRβ signaling results in down-regulation of both GRK2 (27) and the PDGFRβ (7). To determine which widely expressed GRK (GRK2 or GRK5) mediates most of the PDGFRβ regulation in SMCs, we first tested whether the expression level of either of these GRKs was regulated coordinately with the PDGFRβ. Indeed, with prolonged PDGF stimulation that down-regulates the PDGFRβ (7), we observed down-regulation of GRK5, but not GRK2 (Fig. 1). To resolve the apparent paradox between these results and our data showing that endogenous GRK2 regulates PDGFRβs in fibroblasts (8), we compared GRK expression levels in SMCs and fibroblasts.

Consistent with a GRK5-dominant mechanism for PDGFRβ regulation in SMCs, SMCs expressed ~2-fold more GRK5 than fibroblasts, and only ~0.5~0.2-fold as much GRK2 (Fig. 1C).

If vascular SMC GRK5 expression is regulated coordinately with the PDGFRβ in a physiologically meaningful way, then we should expect GRK5 to be up-regulated under pathological conditions that promote PDGFRβ up-regulation too. To test this expectation, we examined GRK5 and PDGFRβ expression in atherosclerosis, a pathologic process involving SMC proliferation and migration in response to myriad cytokines and growth factors (28). The “fibrous cap” of atherosclerotic lesions in the mouse comprises largely SMCs, identified in Fig. 2 by staining for α-SMC actin. Although atherosclerotic and normal arteries demonstrate equivalent α-SMC actin expression per cell, atherosclerotic arteries demonstrate substantially more PDGFRβ and GRK5 expression per α-SMC actin-expressing cell (Fig. 2). Interestingly, unlike GRK5, GRK2 was not up-regulated in these atheroma SMCs (data not shown). The coordinate up-regulation of GRK5 and the PDGFRβ in SMCs of atherosclerotic arteries, along with coordinate down-regulation of GRK5 and the PDGFRβ in cultured SMCs, suggests that GRK5 and the PDGFRβ in SMCs may be functionally related.

To determine possible effects of GRK5 on PDGFRβ activity in SMCs, we began by overexpressing GRK5 in primary rabbit SMCs with a recombinant adenovirus. The prevalence of GRK overexpression was assessed by immunofluorescence microscopy to be 90–100%, as described previously (9). In GRK5-overexpressing SMCs, GRK5 was ~20–30-fold overexpressed,
relative to endogenous GRK5, and PDGFRβ expression was 100 ± 15% of that seen in control SMCs (Fig. 3A and data not shown).

**GRK5 Desensitizes Heptahedral and PDGF Receptors**—Phosphoinositide hydrolysis elicited through SMC heptahedral receptors was clearly desensitized by GRK5 overexpression (Fig. 3B). As we have observed in HEK cells (24), GRK5 inhibited phosphoinositide hydrolysis elicited by endothelin and PAR1 (protease-activated receptor-1). However, contrary to results in HEK cells overexpressing GRK5 (29) or SMCs overexpressing GRK2 (9), GRK5 overexpression also blunted phosphoinositide hydrolysis evoked through thromboxane A2 receptors. This inhibition of Gq-coupled receptor signaling could have been mediated at the level of Goq/11 subunits or by the GRK5 RGS (regulator of G protein signaling) domain (29, 30). Supporting this hypothesis, GRK5 overexpression inhibited thromboxane-evoked phosphoinositide hydrolysis to a greater extent (~70%, p < 0.05). Thus, these levels of GRK5 overexpression appeared to reduce signaling with both receptor-specific and G protein-related mechanisms.

Overexpression of GRK5, like GRK2 (9), also inhibited phosphoinositide hydrolysis effected by the PDGFRβ (the only PDGFR expressed in rabbit aortic SMCs) (32) by 60% (Fig. 3B). Thus, the ability to desensitize both PDGF and heptahedral receptors appears to extend across GRK subtypes. Importantly, this inhibition of PDGFRβ-evoked phosphoinositide hydrolysis did not involve heterotrimeric G proteins. In rabbit SMCs, we found that the PDGFRβ activates Goq, but not Goq/11 (7). Although Gβγ subunits can activate PLC-β (33), we found no evidence of such activation by the PDGFRβ in our SMCs. Treatment of SMCs with pertussis toxin (to inactivate Gi/o) failed to affect PDGF-induced phosphoinositide hydrolysis, but eliminated Gi/o-dependent (34) activation of extracellular signal-regulated kinase (ERK) by lysophosphatidic acid (data not shown). In light of these data, it seemed that overexpression of GRK5 inhibited PDGFRβ-mediated activation of PLC-γ, a tyrosine kinase-dependent event (4).

**PDGF-promoted SMC Migration and Proliferation Are Attenuated by GRK5**—We tested whether overexpression of GRK5 would inhibit PDGF-induced SMC migration, since this process involves not only PLC-γ but also phosphoinositide 3-kinase (10), p125 focal adhesion kinase (32), reactive oxygen species (35), and small G proteins (36, 37). PDGF-promoted migration was 2.2 ± 0.6-fold/basal in vector-infected SMCs, and basal SMC migration was indistinguishable among vector- and GRK5 adenovirus-infected SMC groups (Fig. 3C). However, PDGF-promoted migration was halved by overexpression of GRK5 (Fig. 3C), in a manner congruent with GRK2-overexpressing SMCs (10).

**GRK5 Diminishes PDGFRβ-evoked SMC Thymidine Incorporation and Proliferation**—Although it results from PDGFRβ signaling distinct from that required for migration (4, 10, 38–40), PDGFRβ-evoked SMC thymidine incorporation was also diminished 65–70% in GRK5-overexpressing SMCs, in response to PDGF alone or in synergistic combination with Gq-coupled receptors (Fig. 4A), just as we observed with GRK2.
GRK5, like GRK2 (9), also blunted thymidine incorporation induced by the myriad agonists in fetal bovine serum (in which PDGF plays a critical role) (41). Whereas GRK5 overexpression substantially attenuated thymidine incorporation evoked by the combination of PDGF and Gq-coupled receptor agonists, it failed to affect comparable thymidine incorporation evoked by PDGF plus EGF. Thus, GRK5-mediated desensitization demonstrated substrate specificity for receptor protein-tyrosine kinases in a manner very similar to that observed with GRK2 (10).

To determine whether the same levels of GRK overexpression that inhibited signaling, migration, and thymidine incorporation would also inhibit SMC proliferation, aliquots of the SMC lines used for the former assays were subjected to 12-day

![FIGURE 4. GRK5 overexpression attenuates PDGF-promoted SMC proliferation in a receptor-specific manner. A, SMC thymidine incorporation. Quiescent SMCs infected with the indicated adenovirus were exposed to low-mitogen medium containing vehicle (basal), the indicated agonists at concentrations specified above, 5% fetal bovine serum (FBS), or PDGF plus 0.17 nM EGF. [3H]Thymidine incorporation after 24 h of agonist exposure is plotted as the mean ± S.E. from at least four experiments performed in triplicate. *, p < 0.05 compared with vector-infected SMCs. Uninfected and vector-infected SMCs showed indistinguishable stimulus-induced thymidine incorporation (data not shown). B, lamin ELISA. The indicated number of quiescent SMCs were plated in low mitogen medium and subjected the next day to lamin ELISA. Shown are the means ± S.D. of a single experiment performed in triplicate, representative of eight performed. For A490 versus SMC number, R² = 0.957. C, SMC proliferation. Quiescent, adenovirus-infected SMCs (5 x 10³/well) were exposed to low mitogen medium containing vehicle (basal), 1.5 nM fibroblast growth factor-2 (FGF), or other agonists as specified in Fig. 3. SMC proliferation after 12 days of agonist exposure was assessed by lamin ELISA and plotted as 100 x (stimulated/basal) - 1), means ± S.E. from at least three independent experiments performed in quadruplicate. *, p < 0.05 compared with vector-infected SMCs (by repeated measures analysis of variance).](https://doi.org/10.1074/jbc.M604773200)

![FIGURE 5. GRK5 overexpression reduces PDGFRβ tyrosyl phosphorylation and enhances PDGFRβ/Scap2 association. A, SMCs infected with the indicated adenovirus were rendered quiescent and then stimulated with 2 nM PDGF-BB (37 °C, 10 min) and subjected to IP with anti-PDGFRβ or nonimmune IgG (−). Divided IP samples were subjected to parallel SDS-PAGE and IB, for either phosphotyrosine (pY) or PDGFRβ. Shown are data from a single experiment, representative of three performed. B, band densities for phosphotyrosine in A were normalized to cognate PDGFRβ band densities; each ratio was normalized to that obtained from IPs of PDGF-stimulated SMCs infected with the vector adenovirus (control), to obtain the percentage of control (% of control). Data (mean ± S.E.) are from three independent experiments. *, p < 0.05 compared with control. C, PDGFRβ IPs performed as in A were probed sequentially with anti-PDGFRβ and anti-Scap2 antibodies. Cognate cell lysates (20 μg/lane) were immunoblotted for Scap2. Results are representative of three independent experiments.](https://doi.org/10.1074/jbc.M604773200)
Physiologic expression of GRK5 diminishes PDGFR\(\beta\) phosphorylation.

**A**

Mouse SMC Lysate

| IB:           | WT1 | KO1 | WT2 | KO2 |
|---------------|-----|-----|-----|-----|
| GRK5 genotype|       |     |     |     |
| GRK5          | 82  |     | 82  |     |
| GRK6          | 82  | 82  |     |     |
| GRK2          | 196 | 196 |     |     |
| PDGFR\(\beta\) | 112 | 196 |     |     |
| PLC\(\gamma\) | 112 | 112 |     |     |
| Nonimmune     | 82  |     |     |     |

**B**

Inositol Phosphates (fold/basal)

|         | PDGF | A(\(F\)\)\(^{-}\) |
|---------|------|-----------------|
| Stimulus|      |                 |
|         | WT   | KO              |
|         | 16   | 15*             |

**C**

Thymidine Incorporation (dpm/10\(^2\) cells)

|         | PDGF | EGF |
|---------|------|-----|
| SMCs    |      |     |
| WT      | 20   | 20  |
| KO      | 20   | 20  |

**D**

| IP IgG | PDGFR\(\beta\) (dpm/10\(^2\) cells) |
|--------|-------------------------------------|
| SMC Line | WT | GRK5 KO |
| PDGF     | -  | -       |
| IB: pY   | 195| 195     |
| PDGFR\(\beta\) | 112| 112     |

**FIGURE 6.** Physiologic expression of GRK5 diminishes PDGFR\(\beta\)-evoked phosphoinositide hydrolysis, thymidine incorporation, and PDGFR\(\beta\) tyrosyl phosphorylation. A, aortic SMC lines from individual GRK5-deficient (KO) and littermate WT mice were solubilized, and 20 \(\mu\)g were immunoblotted with (a) murine anti-GRK5/6 (S8) (top) or anti-PLC-\(\gamma\); (b) rabbit anti-GRK2 or -PDGFR\(\beta\); or (c) cognate nonimmune IgG from mouse (bottom) or rabbit (not shown). IBs are representative of three performed for each protein in five SMC lines of each genotype; nonimmune blots revealed no bands corresponding to proteins of interest. B, SMCs were metabolically labeled and stimulated as in Fig. 3. Inositol phosphates expressed as stimulated/basal were averaged among three independent SMC lines of each genotype (\(\pm\)S.E.) (in five experiments/SMC line); means \(\pm\)S.E. are plotted. Basal inositol phosphate values were 0.98 \(\pm\) 0.43 and 0.72 \(\pm\) 0.42 (percentage of conversion units (24)) for WT and KO SMCs, respectively. C, quiescent SMCs were stimulated (or not; basal) with 2 nm PDGF-BB or 1.7 nm EGF and subjected to \(|^{3}H|\)thymidine incorporation assay, as in Fig. 4. Shown are the means \(\pm\)S.E. from four experiments performed with two independent pairs of WT and KO SMC lines. Basal \(|^{3}H|\)thymidine incorporation values were 2 \(\pm\) 1 \(\times\) 10\(^5\) cpmp for both WT and KO SMCs. * \(p < 0.02\) compared with WT (paired analysis). D, SMCs exposed for 5 min (37 °C) to low mitogen medium containing vehicle or 2 nm PDGF-BB were solubilized, and anti-PDGFR\(\beta\) or cognate nonimmune IgG (-) was used for IP. Immunoprecipitates were resolved by SDS-PAGE and immunoblotted serially with the Src homology 2 (SH2) top or anti-PLC-\(\gamma\) (bottom) and then phosphotyrosine (pY) (middle) and plotted as means \(\pm\)S.E. from three independent experiments. Shown are blots from a single experiment, representative of three performed with at least two WT and at least two KO SMC lines each. * \(p < 0.05\) compared with GRK5 KO.
were stimulated and processed for PDGFR purified protein preparations.

GRK5 serine-phosphorylates the PDGFR in intact SMCs. SMCs/H9252 IP and IB just as in Fig. 6 except that IP was performed for the PDGFRβ and then phosphoserine (pSer), not phosphotyrosine. Shown are blots from a single experiment, representative of three performed with at least two WT and at least two KO SMC lines each. Relative densities of pSer bands were quantitated as in Fig. 5. As a result of purified GRK5 activity, this agonist-dependent PDGFRβ seryl phosphorylation increased ~2-fold (Fig. 7B). Purified GRK5 activity showed an even larger relative increase in seryl phosphorylation with PDGFRβs obtained from unstimulated SMCs (~4-fold). However, it should be noted that this "agonist-independent" effect was not independent of PDGFRβ activation. IgG in our immune complex kinase assay dimerizes immunoprecipitated PDGFRβs, and thereby promotes PDGFRβ autophosphorylation/activation (Fig. 7B), which is a prerequisite for GRK-mediated PDGFRβ phosphorylation (7).

In these experiments with vascular SMCs, physiologically expressed GRK5 phosphorylated and desensitized the PDGFR in a manner resembling that of GRK2 expressed physiologically in fibroblasts (8). Indeed, although GRK2 appeared to mediate most of the agonist-induced PDGFRβ seryl phosphorylation in fibroblasts (8), GRK5 mediated most of the agonist-induced

addition, endogenous GRK5 diminished thymidine incorporation evoked by the PDGFRβ (by 56%), but not the EGF receptor (Fig. 6C). Finally, physiologically expressed GRK5 also reduced PDGFRβ tyrosyl phosphorylation, by 35 ± 10% (Fig. 6D). Thus, physiologically expressed GRK5 mediated receptor-specific PDGFRβ desensitization at the level of PDGFRβ autophosphorylation/activation, second messenger signaling, and signaling further downstream from the receptor, and all in a manner congruent with that observed by comparing GRK5-expressing with GRK5-overexpressing SMCs (Figs. 3–5).

For heptahelical receptors, receptor-specific desensitization by GRK5 corresponds to GRK5-mediated phosphorylation of the receptors on Ser or Thr residues (11). To test whether GRK5-mediated PDGFRβ regulation in SMCs followed this paradigm, we assessed PDGF-induced PDGFRβ seryl phosphorylation in WT and grk5−/− SMCs. Indeed, physiologic expression of GRK5 did augment PDGF-induced seryl phosphorylation of the PDGFRβ, by 2.9 ± 0.9-fold (Fig. 7A).

To ascertain that GRK5 itself was responsible for the excess PDGFRβ seryl phosphorylation we observed in GRK5-expressing SMCs, we used purified GRK5 to phosphorylate the partially purified PDGFRβ in vitro (Fig. 7B). In the absence of purified GRK5, we found some PDGF-dependent PDGFRβ seryl phosphorylation (Fig. 7B, lane 2). This PDGFRβ seryl phosphorylation, however, could be attributed to intracellular Ser/Thr kinases, acting before PDGFRβ IP (as in grk5−/− SMCs, in Fig. 7A). As a result of purified GRK5 activity in vitro, this agonist-dependent PDGFRβ seryl phosphorylation increased ~2-fold (Fig. 7B). Purified GRK5 activity showed an even larger relative increase in seryl phosphorylation with PDGFRβs obtained from unstimulated SMCs (~4-fold). However, it should be noted that this "agonist-independent" effect was not independent of PDGFRβ activation. IgG in our immune complex kinase assay dimerizes immunoprecipitated PDGFRβs, and thereby promotes PDGFRβ autophosphorylation/activation (Fig. 7B), which is a prerequisite for GRK-mediated PDGFRβ phosphorylation (7).

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PDGFRβ Regulation by GRK5 in SMCs

FIGURE 8. The PDGFRβ is regulated in SMCs predominantly by GRK5 and not GRK2. WT SMCs were treated with siRNA that targeted the mRNA for no known protein (Control), for GRK2, or for GRK5. Subsequently, the SMCs were metabolically labeled with [3H]inositol and, 72 h after siRNA treatment, stimulated as in Fig. 6 or processed for IB. A, inositol phosphates expressed as fold change in five pairs of WT and KO SMC lines; means ± S.E. are plotted. *, p < 0.05 compared with control SMCs. Basal inositol phosphate values were 2.3 ± 0.8, 2.6 ± 0.4, and 2.3 ± 0.3 (percent conversion units [24]) for SMCs treated with control, GRK2, and GRK5 siRNA, respectively. B, extracts (35 μg of protein) from SMCs treated with the indicated siRNA were subjected to SDS-PAGE and IB. Blots were probed serially for GRK2, GRK5, and actin. Shown are the results of a single experiment, representative of three performed. Serial IB for PDGFRβ showed equivalent PDGFRβ expression in all SMC groups (not shown). C, densitometry of GRK bands was normalized to corresponding actin bands on each blot, and these ratios were normalized to cognate ratios obtained from SMCs transfected with control siRNA, to obtain the percentage of control. Shown are the mean ± S.E. of three experiments. *, p < 0.05 compared with control cells.

PDGFRβ Ser phosphorylation in SMCs. Furthermore, GRK5 augmented PDGFRβ seryl phosphorylation as it does with heptahelical receptors (12), in an agonist-dependent manner and on a rapid time scale congruent with desensitization of second messenger production (seen in Fig. 6B).

Does the predominance of SMC GRK5 in PDGFRβ seryl phosphorylation correlate with a predominance of GRK5 in PDGFRβ desensitization? To address this question, we sought to determine the relative contributions of GRK5 and GRK2 to PDGFRβ regulation in SMCs. To that end, we used siRNA to reduce SMC expression of either GRK2 or GRK5, and assessed the effect of GRK knock-down on phosphoinositide hydrolysis. As we observed with GRK5-null and WT SMCs (Fig. 6B), G protein (fluoroaluminolate)-evoked phosphoinositide hydrolysis was unaffected by changes in GRK expression (Fig. 8A). In contrast, PDGF-evoked phosphoinositide hydrolysis was enhanced (~35%) by reduction in the expression of just GRK5, and not GRK2 (Fig. 8A). Moreover, this GRK-specific difference obtained even though the siRNA-mediated knock-down of GRK2 was somewhat more efficacious than that for GRK5 (Fig. 8, B and C). Consequently, the GRK isoform that regulates PDGFRβ signaling in SMCs predominantly is GRK5, and not GRK2.

GRK5-mediated PDGFRβ Desensitization Involves Shp2—To understand how GRK5-mediated seryl phosphorylation of the PDGFRβ could diminish receptor Tyr phosphorylation (Fig. 7B), we tested whether physiologically expressed GRK5 augmented the association of the PDGFRβ with the phosphatase Shp2, as overexpressed GRK5 did (Fig. 5C). Compared with GRK5-null SMCs, GRK5-expressing SMCs evinced 8 ± 5-fold more PDGF-induced Shp2/PDGFRβ association (range 2–14-fold, in five pairs of WT and KO SMC lines; p < 0.05) (Fig. 9A). Thus, even expressed at physiologic levels, GRK5 serine-phosphorylated the PDGFRβ in a manner that correlated with augmented recruitment of Shp2 to the receptor. Shp2, of course, could serve as an “effector” of GRK5-initiated PDGFRβ desensitization by mediating PDGFRβ dephosphorylation.

How could GRK5-mediated Ser phosphorylation of the PDGFRβ augment Shp2 recruitment to the PDGFRβ? To address this question, we asked whether GRK5 activity affected phosphorylation of PDGFRβ Tyr1009, since phospho–Tyr1009 is the primary PDGFRβ docking site for Shp2 (4). With IgG specific for the Tyr1009-phosphorylated PDGFRβ, we found that Tyr1009 was hyperphosphorylated in GRK5-expressing as compared with GRK5-null SMCs (Fig. 9B). Thus, there was a greater prevalence of Tyr1009-phosphorylated PDGFRβ in GRK5-expressing SMCs, and consequently a greater prevalence of PDGFRβs capable of recruiting Shp2.

This finding demonstrates that the GRK5-mediated reduction in overall PDGFRβ tyrosyl phosphorylation is site-specific. Indeed, although GRK5 activity enhanced phosphorylation of PDGFRβ Tyr1009, it substantially diminished phosphorylation of PDGFRβ Tyr1021 (Fig. 9C). PDGFRβs from GRK5-expressing SMCs demonstrated 9 ± 3-fold less phospho–Tyr1021 than PDGFRβs from GRK5-null SMCs (p < 0.05). This GRK5-associated reduction in PDGFRβ phospho–Tyr1021 would be expected to reduce PLC-γ1/PDGFRβ association and consequent PLC-γ1-mediated phosphoinositide hydrolysis (4), just as we observed with these SMCs in Fig. 6B. Such site-specific reduction in PDGFRβ tyrosyl phosphorylation probably explains why overall PDGFRβ tyrosyl phosphorylation is only modestly reduced in GRK5-expressing (as compared with GRK5-null) SMCs (Fig. 6D).
To confirm that physiologic GRK5 expression was responsible for the differences in PDGFR phosphorylation and signaling we observed between GRK5-expressing and -null SMCs, we took two approaches. First, as described above, we obtained congruent results from five pairs of WT and cognate GRK5-null SMC lines. Second, we used our GRK5 adenovirus to express GRK5 at 10^4/10^8% of WT levels in GRK5-null SMCs, to test whether "rescuing" GRK5 expression would convert a GRK5-null to a WT SMC phenotype (Fig. 10). For this purpose, we assayed PDGF-induced phosphorylation of the PDGFR at Tyr1021 and found that GRK5 "rescue" expression in GRK5-null SMCs reduced phosphorylation of PDGFR Tyr1021 by 7±3-fold (p<0.05) (Fig. 10), and enhanced PDGFR/β/β2 association by ~3-fold (Fig. 10). These results were remarkably congruent, of course, with those obtained by comparing WT and grk5^-/- SMCs in Fig. 9. Thus, whether expressed endogenously or heterologously, physiologic levels of GRK5 expression mediate PDGFR desensitization.

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Thus far, we have correlated GRK5-mediated seryl phosphorylation of the PDGFR with diminished PDGFR tyrosyl phosphorylation, desensitization of PDGFR-β-evoked SMC signaling, and enhancement of PDGFR/β/β2 association. To demonstrate more directly that GRK5-mediated PDGFRβ regulation involves Shp2, we compared GRK5-mediated desensitization of the WT PDGFRβ and Y1009F mutant PDGFRβ, which recruits Shp2 poorly (42) (Fig. 11, A and B). To compare these PDGFRβs under conditions of comparable GRK5 levels (Fig. 11D), we used HEK cells (which lack endogenous PDGFRβs) (27). We used phosphorylation of PDGFRβ Tyr1021 and Tyr740 as read-outs for PDGFRβ activation. Correlating again with enhancement of Shp2/PDGFRβ association (Fig. 11B), increased cellular GRK5 activity substantially reduced...
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A

| 293 Cell Transfection | WT PDGFRβ | Y1009F PDGFRβ |
|-----------------------|------------|---------------|
| Vector (wt)           | ±          | ±             |
| GRK5 (high)           | ±          | ±             |
| PDGF                  | 195        | 195           |

IB:

- pY-1021: 112 kDa
- pY-740: 59 kDa
- Shp2: 59 kDa
- PDGFRβ: 195 kDa

B

GRK5 Level
- Native
- High

PDGFRβ Construct

Shp2 / PDGFRβ (% of control)

C

PDGFRβ Construct

p-Tyr / PDGFRβ (% of control cells)

D

PDGFRβ Construct

GRK5 Level
- Native
- High

Lyse IB IgG
- Anti-GRK5
- Non

FIGURE 11. GRK5-mediated desensitization of the PDGFRβ requires intact Shp2/PDGFRβ association. HEK 293 cells were transfected with plasmids encoding a human N-terminal FLAG-tagged PDGFRβ construct (WT or Y1009F), GRK5 (‘high’ GRK5 level), or no protein (Vector, ‘native’ GRK5 level). Cells were exposed to medium containing vehicle (−) or 2 nM PDGF-BB (+) for 5 min (37 °C), and then lysed and subjected to IP of the indicated PDGFRβ construct. Divided IP samples were subjected to parallel SDS-PAGE and sequential IB (with intervening membrane stripping) for the PDGFRβ and then either the PDGFRβ phosphorylated at Tyr1021 (pY-1021), the PDGFRβ phosphorylated at Tyr740 (pY-740), or Shp2. A, blots from a single experiment are displayed and represent three experiments performed with similar results. HEK cells transfected without a PDGFRβ construct yielded no signals on these blots, and all cell lines expressed equivalent levels of Shp2 (data not shown). B, quantitation of Shp2/PDGFRβ association. Shp2 band densities were normalized to cognate PDGFRβ band densities; each ratio was normalized.
GRK5 Potentiates PDGFRβ-induced Activation of Src, but Not ERK—The association of Shp2 with the PDGFRβ is believed to activate Shp2 (4). Consequently, since GRK5 activity in SMCs augments Shp2/PDGFRβ association, we expected to observe not only reduced PDGFRβ tyrosyl phosphorylation but also other evidence of enhanced PDGF-induced Shp2 activity. To test this expectation, we examined PDGF-promoted ERK1/2 and Src activation, which can be mediated by Shp2 (4, 44). Despite large differences in PDGFRβ/Shp2 association (Fig. 9), GRK5-expressing and -null SMCs demonstrated equivalent activation of ERK-1 and -2 within 5 min of PDGF stimulation (data not shown). This finding is consonant with data from Shp2-deficient fibroblasts. Even the absence of Shp2 does not diminish PDGFRβ-promoted ERK activation within 5 min of PDGF stimulation (44). However, GRK5-expressing SMCs demonstrated 1.9-fold more Src activation than GRK5-null SMCs (Fig. 14, A and B), even though GRK5 activity did not affect PDGFRβ autophosphorylation on its docking site for Src (Tyr579) (Fig. 14 C). Thus, GRK5-mediated seryl phosphorylation of the PDGFRβ desensitizes signaling selectively. Although PLC-γ1 and perhaps other pathways promoting SMC migration and proliferation are desensitized, Src signaling is augmented. In this way, GRK5-mediated phosphorylation of the PDGFRβ mirrors GRK-mediated phosphorylation of heptahedral receptors, a process which desensitizes signaling through

FIGURE 12. Reduction of Shp2 expression diminishes GRK5-mediated desensitization of the PDGFRβ in SMCs. WT and grk5−/− (KO) SMCs were treated with siRNA that targeted either no known mRNA (control or CTL), or the mRNA for Shp2. Subsequently, SMCs were metabolically labeled with [3H]inositol and, 72 h after siRNA treatment, stimulated as in Fig. 6 or processed for IB. A, extracts (30 μg of protein) from SMCs treated with the indicated siRNA were subjected to SDS-PAGE and IB. Blots were probed serially for Shp2 and tubulin. Shown are the results of a single experiment, representative of three performed. Subsequent PDGFRβ IB demonstrated that Shp2 siRNA did not affect PDGFRβ expression (not shown). B, densitometry of Shp2 bands was normalized to corresponding tubulin bands on each blot, and these ratios were normalized to cognate ratios obtained from WT SMCs transfected with control siRNA, to obtain the "percentage of WT control." Shown are the mean ± S.E. of three experiments. *, p < 0.05 compared with WT control cells. C, inositol phosphates obtained from stimulated SMCs were divided by inositol phosphates obtained from corresponding unstimulated SMCs (-fold/basal); these ratios were normalized within each experiment to the cognate -fold/basal value obtained for PDGF-stimulated WT SMCs treated with control siRNA (6-fold/basal), to obtain the "percentage of WT control." Potted are means ± S.E. from six independent experiments (two with each of the three WT/KO SMC pairs). *, p < 0.05 compared with cognate control siRNA-treated SMCs. Basal inositol phosphate values were 5 ± 3.5 ± 2.7 ± 1, and 7 ± 2 (percentage of conversion units (24)) for WT and KO SMCs treated with control and Shp2 siRNA, respectively.
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G proteins but activates signaling through ERKs (11, 19, 20), and possibly Src (45, 46). A provisional model for GRK5-mediated regulation of the PDGFRβ/H9252 is presented in Fig. 15.

DISCUSSION

This study demonstrates for the first time that the PDGFRβ is phosphorylated and desensitized by GRK5, a widely expressed kinase previously known only to regulate a multitude of heptahelical receptors (11). Moreover, GRK5 mediates the preponderance of PDGF-induced seryl phosphorylation and desensitization of the PDGFRβ/H9252 in SMCs, and reduces overall PDGFRβ tyrosyl phosphorylation in a manner that is highly site-specific. Whether assessed as second messenger production, migration, thymidine incorporation, or proliferation, PDGFRβ-promoted SMC activity was reduced in a receptor-specific manner by GRK5 activity. Although GRK5-mediated

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**FIGURE 14.** GRK5 activity augments PDGF-evoked activation of Src. A, quiescent GRK5-null and WT SMCs were stimulated (or not) with 2 nM PDGF-BB for 10 min (37 °C) and lysed; 20 μg of protein from each cell group underwent parallel SDS-PAGE and IB for either activated Src, phosphorylated on Tyr\textsuperscript{pS} (pY-416), or total Src (bottom). B, Src pY-416 band density was normalized to cognate Src band density, and ratios were normalized to those obtained for PDGF-stimulated GRK5-null SMCs to obtain the “percentage of GRK5 KO.” Shown are means ± S.E. from eight experiments performed with four paired KO and WT SMC lines. *, p < 0.05 compared with GRK5-null. C, WT and GRK5 KO SMCs were stimulated (or not) with PDGF-BB and subjected to PDGFRβ IP, SDS-PAGE, and IB as in Fig. 9. Blots were probed serially for the PDGFRβ and then the PDGFRβ phosphorylated on Tyr\textsuperscript{pS} (one of two Src docking sites (4)). Shown are the results of a single experiment, representative of three performed.

**FIGURE 15.** Proposed scheme for GRK5-mediated regulation of the PDGFRβ. A, the agonist-activated, dimerized, and autophosphorylated PDGFRβ is schematically depicted in a caveola, into which it appears to migrate after activation (51). The PDGFRβ, which can activate heterotrimeric G, (7), allosterically activates GRK5, which may be localized to caveolae by its binding to caveolins-1 (49). Once activated, the GRK5 phosphorylates the activated PDGFRβ on seryl residues. B, by as yet unknown mechanisms, GRK5-mediated phosphorylation of PDGFRβ seryl residues leads to enhanced phosphorylation of PDGFRβ Tyr\textsuperscript{pS} (one of two Src docking sites (4)). Shown are the results of a single experiment, representative of three performed. C, because GRK5 activity on the PDGFRβ augments Shp2/PDGFRβ association and Shp2 activation without affecting the ability of PDGFRβ to recruit Src (Fig. 14C), PDGF-induced Src activation is enhanced in GRK5-expressing SMCs. With increased levels of activated Shp2 recruited to the PDGFRβ, there is greater Src-mediated dephosphorylation of Src at its (autoinhibitory) phospho-Tyr\textsuperscript{pS} (Figs. 9–11)); the resulting decrease in PDGFRβ activity is symbolized by removal of the asterisk from R\textsuperscript{*}.
PDGFR\(\beta\) desensitization critically involves the PDGFR\(\beta\) phosphatase Shp2, GRK2-mediated PDGFR\(\beta\) desensitization does not.

As a regulator of PDGFR\(\beta\) activity, GRK5 might seem unusual. With rare exception (47), substrates for GRK5 are all heptahelial receptors that activate heterotrimeric G proteins. However, we have shown that, like many heptahelial receptors, the PDGFR\(\beta\) itself activates Go, in SMC membranes, even in the absence of ATP (7) (an approach that precludes G\(_i\)-coupled sphingosine-1-phosphate receptor activation by PDGFR\(\beta\)-activated sphingosine kinase) (48). As a result, the PDGFR\(\beta\) cytoplasmic tail conformation that activates G\(_i\) might reasonably be expected to activate GRK5 allosterically, too. Moreover, the PDGFR\(\beta\) and GRK5 could be expected to share subcellular localization in quiescent cells. GRK5 resides primarily on membranes (11) and binds to caveolin-1 (49), whereas the PDGFR\(\beta\) resides in caveolae (50), at least transiently (51).

A role for GRK5 in regulating the PDGFR\(\beta\) might also seem improbable, however, from the perspective of studies that employed purified proteins to examine inhibition of GRK5 activity. Consequent to PDGFR\(\beta\)-mediated PLC-\(\gamma\) activation, intracellular [Ca\(^{2+}\)] rises, and protein kinase C isoforms are activated (4). In preparations with purified proteins, Ca\(^{2+}\)/calmodulin binding and protein kinase C-mediated phosphorylation of GRK5 have been shown to inhibit the ability of GRK5 to associate with membrane-bound substrates (11). Moreover, whereas protein kinase C-mediated GRK2 phosphorylation relieves Ca\(^{2+}\)/calmodulin-mediated inhibition of GRK2 (52), it actually inhibits GRK5 catalytic activity (11). These mechanisms may underlie the apparent inability of GRK5 to regulate angiotensin II AT\(_1\) receptor signaling in mouse myocardium (53). Nevertheless, in intact SMCs, our results indicate that despite possible attenuation by Ca\(^{2+}\)/calmodulin- and protein kinase C-mediated inhibition, the net activity of physiologically expressed GRK5 is clearly sufficient to achieve agonist-dependent PDGFR\(\beta\) phosphorylation and desensitization.

By what mechanism might GRK5-mediated seryl phosphorylation of the PDGFR\(\beta\) enhance phosphorylation of the PDGFR\(\beta\) Tyr\(^{1009}\), and thereby PDGFR\(\beta\)/Shp2 association? Although the Ser/Thr kinase GRK5 could not plausibly phosphorylate PDGFR\(\beta\) Tyr\(^{1009}\) directly, GRK5-mediated Ser phosphorylation could enhance Tyr\(^{1009}\) phosphorylation indirectly. The PDGFR\(\beta\) sequence (54) surrounding Tyr\(^{1009}\) includes DTS\(^{1005}\)SVLY\(^{1009}\), where Ser\(^{1005}\) and Ser\(^{1006}\) are highly plausibly GRK phosphorylation sites (11). If GRK5 were to phosphorylate either Ser\(^{1005}\), Ser\(^{1006}\), or both, the resulting phosphoseryl residue(s) would increase the negative charge N-terminal to Tyr\(^{1009}\), and thereby possibly enhance phosphorylation of Tyr\(^{1009}\) by the PDGFR\(\beta\) Tyr kinase (55). (The mouse sequence is identical to human over a 19-amino acid stretch surrounding Tyr\(^{1009}\) but the mouse tyrosyl residue is numbered 1008.) In light of this proposed scheme, it is of interest to note that metabolically labeled endothelial cells phosphorylate PDGFR\(\beta\)s on seryl residues distinct from (and C-terminal to) Ser\(^{1005}\) and Ser\(^{1006}\) (39). However, these endothelial cell data may not be relevant to cells (like SMCs) in which GRK5 is the dominant GRK. GRK2 appears to be the dominant GRK in endothelial cells (56), and we have shown that GRK2 phosphor- ylates the PDGFR\(\beta\) on Ser\(^{1104}\), perhaps among other sites (8). The PDGFR\(\beta\) residue(s) phosphorylated by GRK5 are as yet unidentified.

Although GRK5-mediated PDGFR\(\beta\) seryl phosphorylation reduces autophosphorylation at PDGFR\(\beta\) Tyr\(^{1021}\) (the docking site for PLC-\(\gamma\) (4)) by \(\sim\)9-fold (Fig. 9), the mechanism by which GRK5 reduces this site-specific Tyr phosphorylation remains somewhat uncertain. We have correlated reduction in PDGFR\(\beta\) Tyr\(^{1021}\) phosphorylation with the GRK5-mediated increase in PDGFR\(\beta\)/Shp2 association. However, the ability of Shp2 to dephosphorylate PDGFR\(\beta\) Tyr\(^{1021}\) is relatively poor in studies performed with purified proteins (57). Could this apparent site-specific relative “deficiency” in Shp2-mediated PDGFR\(\beta\) dephosphorylation result from the absence of key Shp2 or PDGFR\(\beta\) binding partners in the purified protein assay? Such a possibility is suggested by our data in SMCs. It remains to be determined whether accessory proteins facilitate direct Shp2-mediated dephosphorylation of PDGFR\(\beta\) Tyr\(^{1021}\) or whether Shp2 dephosphorylates PDGFR\(\beta\) Tyr\(^{1021}\) indirectly, through one or more of these accessory proteins.

The discovery that GRK5 regulates the PDGFR\(\beta\) in SMCs suggests that GRK5, like the PDGFR\(\beta\) itself (1, 3), may play a role in the pathogenesis of atherosclerosis. The up-regulation of GRK5 in SMC-like atheroma cells supports this concept (Fig. 2). From what we have learned with GRK5-deficient and WT SMCs in this study, we would expect that GRK5 activity should attenuate atherosclerosis. Whether this possibility obtains in vivo, of course, remains to be determined.

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