Modulation of α₇-integrin-mediated adhesion and expression by platelet-derived growth factor in vascular smooth muscle cells

Jun-Tzu Chao,¹ Luis A. Martinez-Lemus,¹ Stephen J. Kaufman,² Gerald A. Meiningner,¹ Kenneth S. Ramos,³ and Emily Wilson¹

¹Division of Vascular Biology, Cardiovascular Research Institute, The Texas A&M University System Health Science Center, College Station, Texas; ²Department of Cell and Structural Biology, University of Illinois, Urbana, Illinois; and ³Department of Biochemistry and Molecular Biology, University of Louisville, Louisville, Kentucky

Submitted 23 March 2005; accepted in final form 2 November 2005

Chao, Jun-Tzu, Luis A. Martinez-Lemus, Stephen J. Kaufman, Gerald A. Meiningner, Kenneth S. Ramos, and Emily Wilson. Modulation of α₇-integrin-mediated adhesion and expression by platelet-derived growth factor (PDGF) on α₇-integrin expression and VSMC adhesion and migration. The α₇-integrin gene was determined by real-time RT-PCR and mRNA levels were determined by fluorescence-activated cell sorting analysis. PDGF increased α₇ cell surface protein expression (12 and 24 h: 3.6 ± 0.4-fold, P < 0.05 vs. control) and mRNA levels (24 h: 3.1-fold, P < 0.05 vs. control) in a time-dependent manner. Actinomycin D and SB-203580 attenuated PDGF-induced increases in α₇-integrin, indicating the involvement of de novo mRNA and protein synthesis. Treatment with the MAPK inhibitors PD-98059, SP-600125, and SB-203580 attenuated PDGF-induced increases in α₇-integrin. PDGF did not alter migration on laminin, and there was no effect of the anti-α₇-integrin function-blocking antibody on basal or PDGF-stimulated migration. Immunofluorescence imaging revealed an increase in α₇-integrin distribution along the stress fibers. Together, these observations indicate that PDGF enhances α₇-integrin expression in VSMC and promotes α₇-integrin-mediated adhesion to laminin.

vascular injury; laminin; mitogen-activated protein kinase

INTEGRINS PLAY ESSENTIAL ROLES in cellular functions such as migration, adhesion, differentiation, and proliferation. Upon binding to the extracellular matrix (ECM), integrins undergo conformational changes in the cytoplasmic domain that initiate recruitment of adaptor and cytoskeletal proteins and signaling molecules. Along with the activation of the signaling molecules, integrins are able to transduce signals from extracellular stimuli into the nucleus that may alter gene expression. Consequently, integrins modulate cellular phenotypic changes (29).

In this regard, integrins and growth factors share many common elements in their respective signal transduction pathways (14, 34), and significant cross talk and interaction between growth factor and integrin signaling have been shown previously (14, 35, 36, 41). For example, α₁β₁ and α₅β₁-integrins mediate a mechanically induced mitogenic response in vascular smooth muscle cells (VSMC) by initiating the secretion of platelet-derived growth factor (PDGF) (40). Whereas integrins are known to be involved extensively in the modulation of growth factor signaling, the role of growth factor in modulating integrin expression is not well defined.

PDGF, one of the major growth factors present in blood vessels, plays a critical role in several vascular pathologies. In response to arterial injury such as balloon angioplasty, PDGF stimulates proliferation and migration of VSMC (25). PDGF modulates these cellular functions by activating signaling cascades, such as phosphatidylinositol 3-kinase, Ras, Raf, and mitogen-activated protein kinases (MAPK), including ERK, JNK, and p38 (17, 26). Through these signaling pathways, PDGF modulates the expression of a number of genes that contribute to vascular remodeling. Among the genes regulated are ECM proteins such as fibronectin, laminin-5, and osteopontin in VSMC (22, 26, 39). In addition, PDGF modulates the expression of integrins, such as α₁β₁-integrin complex and α₅-integrin subunit in VSMC (19). However, little is known regarding the effect of PDGF on α₇-integrin subunit expression.

α₅β₁-integrin is a laminin receptor present in skeletal and cardiac muscle (37). In skeletal muscle, α₅β₁-integrin stabilizes the structure of myofibers by providing linkages between laminin in the ECM and the actin cytoskeleton. Increased expression of α₇-integrin subunit is observed in patients with muscular dystrophy caused by dystrophin deficiency (18) and in mice with sarcoglycan or dystroglycan deficiency (3). Expression of α₇-integrin subunit levels also is seen in regenerating myofibers in rats with transection-induced muscle injury (20). The above-described findings suggest that α₇-integrin subunit plays an essential role in the structural stability of myofibers and in the skeletal muscle response to injury. Furthermore, studies by Babbbit et al. (4) demonstrated increased expression of α₇-integrin subunit in a rat model of pressure overload left ventricular hypertrophy. These studies suggest a role of α₇-integrin subunit in the adaptive response of myocardium to pressure overload (4).

The role of α₇-integrin subunit in VSMC is less well characterized. The presence of α₇-integrin subunit in VSMC was reported by Yao et al. (42). Our group demonstrated...
Previously (10) the pathological significance of α7-integrin subunit in VSMC by showing increased α7-integrin subunit expression and adhesion to laminin in VSMC in a chemical model of atherosclerosis. Nonetheless, the physiological regulation and function of α7-integrin subunit is not clear. Thus the objective of this study was to determine the effect of PDGF on α7-integrin subunit expression and whether changes in gene expression induced by PDGF correspond to the functional alterations of VSMC. Using PDGF-treated VSMC as an in vitro model to study vascular injury, we investigated the potential modulation of α7-integrin subunit expression and the regulatory mechanisms responsible for these changes. The role of α7-integrin subunit in VSMC adhesion and migration also was investigated.

MATERIALS AND METHODS

Cell culture and treatment. VSMC were isolated by enzymatic digestion of aortas from Sprague-Dawley rats as previously described (11). Cells were grown in medium 199 (Invitrogen, Carlsbad, CA) supplemented with 10% FBS (HyClone, Logan, UT), 2 mmol/l glutamine, 100 units of penicillin, and 100 μg of streptomycin (Invitrogen) in 5% CO2-95% air at 37°C. Recombinant human PDGF-BB, epidermal growth factor (EGF), fibroblast growth factor (FGF), and transforming growth factor (TGF)-β were purchased from PeproTech (Rocky Hill, NJ). Cells were used between passages 4 and 10 to verify that passage number is not a primary factor in α7-integrin subunit expression. VSMC were quiesced by serum starvation (0.5% FBS M199) for 48 h before PDGF treatment (10 ng/ml for 0, 4, 12, and 24 h). At the end of treatment, cells were processed for either immunofluorescence imaging or adhesion assays. Immunofluorescence imaging was performed using a laser scanning confocal system (Ultima-Z312; Meridian Instruments, Okemos, MI) connected to an inverted microscope (Zeiss 135). Images were collected with a ×63 oil objective (numerical aperture 1.4). Excitation of the fluorophores was achieved using the 488- and 647-nm lines of an argon-krypton laser combination.

Immunofluorescence imaging. After PDGF treatment, VSMC were fixed with 2% paraformaldehyde in PBS for 15 min at room temperature, followed by two washes with PBS to remove the excess paraformaldehyde. Cells were then incubated with H36 anti-α7-integrin antibody overnight at 4°C (1:350 dilution) and washed six times with a solution containing 15 mmol/l sodium citrate, 150 mmol/l sodium chloride, and 0.05% Triton X-100. After being washed, cells were incubated with the secondary antibody, Alexa 488-conjugated goat anti-mouse IgG (5 μg/ml), for 30 min at room temperature (Molecular Probes, Corvallis, OR). Alexa 647-conjugated phalloidin (66 mmol/l; Molecular Probes) was used to stain actin after the incubation with the secondary antibody. Cells also were incubated with mouse IgG isotype as the primary antibody and with the secondary antibody Alexa 488-conjugated goat anti-mouse IgG as a negative control. An additional negative control consisted of incubation with secondary antibody only. Immunofluorescence imaging was performed using a laser scanning confocal system (Ultima-Z312; Meridian Instruments, Okemos, MI) connected to an inverted microscope (Zeiss 135). Images were collected with a ×63 oil objective (numerical aperture 1.4). Excitation of the fluorophores was achieved using the 488- and 647-nm lines of an argon-krypton laser combination.

Adhesion assay. VSMC adhesion to laminin-1 was determined using CytoMatrix cell adhesion strips from Chemicon (Temecula, CA) following the manufacturer’s protocol. Cells were quiesced for 48 h before PDGF treatment. Control and PDGF-treated VSMC were detached with Versene (1:5,000 dilution) at 37°C for 5 min to prepare the cell suspension. Cells (106 cells/ml) were added to laminin-1-coated wells and incubated at 37°C for 1 h. Nonadherent cells were removed by washing (twice) gently with Dulbecco’s PBS (DPBS) in the absence of Ca2+ and Mg2+. The adhered cells were solubilized with acetate buffer (pH 4.5) and quantified by determining the absorbance at 550 nm. The effect of blocking α7-integrin subunit was tested by preincubating the cells with mouse anti-rat α7-integrin antibody (37) (O26, 1:350 dilution) at room temperature for 30 min before the assay. Cells attached to the wells coated with BSA were used to adjust for nonspecific binding (negative control), which was <5% of maximal binding.

Alternative adhesion assays also were performed by using wells coated with anti-α5– (provided by S. J. Kaufman), anti-α7–, and anti-β1–integrin antibodies (BD Pharmingen, San Diego, CA). Wells were coated with anti-α7-integrin antiseraum at a 1:500 dilution; anti-α5– and anti-β1–integrin antibodies were used at 1 μg/ml overnight at 4°C. The excess antibody was removed by washing with DPBS before use. Control and PDGF-treated VSMC were detached as described previously. Cells (106 cells/ml) were added to antibody-coated wells and incubated at 37°C for 1 h. Nonadherent cells were removed by washing (twice) gently with DPBS with Ca2+ and Mg2+, followed by staining with 0.1% crystal violet for 5 min. Excess stain was removed by washing with DPBS in the absence of Ca2+ and Mg2+. The adhered cells were solubilized and quantified as described previously. Effect of blocking α7-integrin subunit was tested by preincubating the cells with mouse anti-rat α7-integrin antibody (37) (O26, 1:350 dilution) at room temperature for 30 min before the assay. Cells attached to the wells coated with BSA were used to adjust for nonspecific binding (negative control), which was <5% of maximal binding.

Migration assay. Cell migration on laminin-1 was determined using a 48-well chemotaxis chamber and polystyrenepyrrolidone-free
polycarbonate filters with pores of 8 μm (Neuro Probe, Gaithersburg, MD) modified from those described by Partridge et al. (30). Each filter was precoated with 20 μg/ml laminin-1 (Sigma) and blocked with 1 mg/ml BSA before the assay was performed. Briefly, control and PDGF-treated VSMC were prepared and detached as described for the adhesion assay. A 28-μl volume of 10% FBS M199 was used as the chemoattractant placed in each well of the lower chamber. A 40-μl volume of cell suspension containing 30,000 cells was placed in the upper chamber and incubated for 4 h at 37°C in a 5% CO2 incubator. The filter was removed, fixed in methanol, and stained (0.1% amido black, 30% methanol, and 10% acetic acid). Staining intensity of migrated cells was determined by densitometry using Multi-Analyst software version 1.0 (Bio-Rad, Hercules, CA).

Statistical analysis. Results are presented as means ± SE of at least three independent experiments. Statistical differences were analyzed using either ANOVA or unpaired t-test with P < 0.05 considered significant. All analyses were performed using GraphPad Prism version 4.02 software for Macintosh (GraphPad Software, San Diego, CA). ANOVA with Tukey’s multiple comparison posttest was performed.

RESULTS

PDGF increases α7-integrin subunit expression in a time-dependent manner. We first examined the time-related effect of PDGF on α7-integrin subunit mRNA and protein expression in VSMC. Cells incubated at the time points indicated without PDGF were used as control. As shown in Fig. 1A, a time-dependent increase of α7 mRNA levels was observed. A significant increase of α7-integrin subunit mRNA was noted at 24 and 48 h after PDGF treatment (3.1- and 4.4-fold, respectively; P < 0.05 vs. control). The surface expression of α7 protein levels also was increased significantly with PDGF treatment compared with control (12 h, 3.30-fold; 24 h, 3.6-fold; and 48 h, 6.7-fold) (Fig. 1B). Although a more significant increase in α7-integrin subunit levels was observed at 48 h, more cell loss also was noted (data not shown), suggesting that long-term maintenance of the cells in low serum was detrimental. Thus, for the following studies, cells were treated with PDGF for 24 h.

Effect of growth factors on α7-integrin subunit expression. To extend the results of FACS and real-time RT-PCR analyses and to determine whether increases in α7-integrin were specific for PDGF, we quiesced VSMC for 48 h and then treated cells with 10 ng/ml PDGF, EGF, FGF, or TGF-β for 24 h. Total protein was isolated, and immunoblot analyses were performed as described. Figure 2A and B shows that only PDGF treatment significantly increased α7-integrin protein level (42 ± 2.7%; P < 0.05 vs. control), whereas no statistically significant difference in α7 protein level was seen in TGF-β-, EGF-, or FGF-treated cells. When comparing the expression of α-actin or GAPDH between control and growth factor-treated cells (Fig. 2A, b and c), we observed no difference.

PDGF modulates α7-integrin subunit expression at transcriptional level. To determine whether the observed changes induced by PDGF were mediated at the transcriptional or translational level, we treated cells with PDGF for 24 h in the presence of 2 μg/ml of either actinomycin D or cycloheximide. mRNA levels of α7-integrin subunit were determined using real-time RT-PCR. As shown in Fig. 3, both actinomycin D and cycloheximide treatments prevented the increase of α7-integrin subunit mRNA level by PDGF (0.9- and 0.8-fold, respectively; P < 0.05 vs. PDGF). These results suggest that increased α7-integrin mRNA is regulated at the transcriptional level and that new protein synthesis is required in this process.

Signaling pathways contributing to PDGF-induced α7-integrin subunit expression. To elucidate the signaling pathways that contribute to PDGF-induced increases in α7-integrin subunit expression, we determined the effects of the MAPK inhibitors PD-98059 (30 μmol/l), SP-600125 (5 μmol/l), and SB-203580 (5 μmol/l) using real-time RT-PCR. As shown in Fig. 4A, all three MAPK inhibitors attenuated PDGF-induced increases in α7-integrin subunit mRNA levels, albeit to different degrees (PD-98059, 55.8 ± 11.3%; SB-203580, 66.7 ± 14.5%; and SP-600125, 81.2 ± 10.3%; n = 4). In comparison, PD-98059 and SP-600125, but not SB-203580, attenuated increased α7-integrin subunit protein levels by PDGF (PD-98059, 62 ± 6.7%; SP-600125, 72.1 ± 11%; n = 5) (Fig. 4B).

PDGF increases VSMC adhesion but not migration on laminin-1. To determine the extent to which PDGF-mediated increases in α7-integrin subunit alter cellular functions, we determined cell adhesion to and migration on laminin-1. As shown in Fig. 5A, PDGF treatment significantly increased the adhesion of VSMC to laminin-1 (42.4 ± 6.3%; P = 0.0037 vs. control). When PDGF-treated VSMC were incubated with a function-blocking anti-α7-integrin antibody before the adhe-
sion assay, adhesion to laminin-1 was partially but significantly decreased (23 ± 7%;  P < 0.0219 vs. PDGF). These results indicate that PDGF treatment (24 h) increases the adhesion to laminin and that this increase is partially mediated by α7-integrin subunit. Mouse IgG isotype was used as a negative control, and no significant difference was observed compared with PDGF-treated VSMC (data not shown).

To exclude the possibility that PDGF modulates the expression of other integrins leading to increased adhesion, we performed the adhesion assay using control and PDGF-treated cells on wells coated with anti-α7-integrin antibody, anti-α5-integrin antibody, or anti-β1-integrin antibodies. Adhesion of PDGF-treated VSMC to anti-α7-integrin antibody-coated wells increased by ~53% (  P < 0.01 vs. control; Fig. 5B). Minimal adhesion to anti-α5-integrin antibody-coated wells was observed in either control or PDGF-
treated cells. Among the three integrins examined, anti-α7-integrin antibody appeared to mediate adhesion more effectively in both control and PDGF-treated cells (P < 0.05 vs. α5 and β1). Minimal adhesion to wells coated with IgG or uncoated wells was observed.

We also determined the effect of PDGF on VSMC migration on laminin-1. As shown in Fig. 5C, PDGF-treated cells displayed no migratory advantage on laminin-1 compared with control. Also, no effect of incubation with anti-α7-integrin antibody was observed for either PDGF-treated or control cells. In contrast, incubation with anti-α1-integrin antibody with both control and PDGF-treated VSMC reduced the migration on laminin-1 significantly.

Localization of α7-integrin in PDGF-treated VSMC. We next examined the distribution pattern of α7-integrin subunit in PDGF-treated or control cells. In Fig. 6, the immunofluorescence of α7-integrin subunit is shown on green (A, C, D, and F), F-actin is shown in red (B and E), and the overlap of α7-integrin subunit and F-actin is shown in yellow (C and F). Punctate staining of α7-integrin subunit in the cells and on the plasma membrane was found in both control and PDGF-treated cells (Fig. 6, A and D). PDGF treatment increased the intensity of α7-integrin subunit staining, which indicates the increased levels of α7-integrin compared with control. This result is consistent with the results of FACS and immunoblot analyses. Spatial association of α7-integrin subunit with F-actin was observed mainly at the periphery of the control cells (Fig. 6C). In PDGF-treated cells, α7-integrin subunit staining localized along with the stress fibers (Fig. 6, E and F). Cells also were incubated with mouse IgG isotype as the primary antibody, with the secondary antibody Alexa 488-conjugated goat antimouse IgG as a negative control. An additional negative control consisted of incubation with secondary antibody only. Both negative controls showed no fluorescence (data not shown).

DISCUSSION

α7β1-Integrin is one of the laminin receptors present in cardiac, skeletal, and vascular smooth muscle. The significance of α7β1-integrin expression is demonstrated by increased expression in patients with muscular dystrophy and murine models of muscular dystrophy caused by dystrophin deficiency. Previous findings by others suggest that in muscular dystrophy, enhanced expression of α7-integrin subunit compensates for the loss of the dystrophin glycoprotein complex, another laminin receptor present in skeletal muscle that stabilizes myofibers (3, 18). The compensatory role of α7-integrin subunit is underscored by the observation that overexpression of α7-integrin subunit rescues the lethal muscular dystrophy phenotype in mice (7). Whereas the function of α7-integrin subunit has been characterized in skeletal muscle, the role of α7-integrin subunit in VSMC has not been explored.

Our group (10) previously demonstrated increased expression of α7-integrin subunit in a chemical model of atherosclerosis. In our present study, a significant increase of α7-integrin subunit expression was observed in VSMC with prolonged treatment (24 and 48 h) of PDGF. We also investigated the regulatory mechanisms by which PDGF modulates the expression of α7-integrin subunit.

To determine whether increased α7-integrin expression is a universal response to growth factor stimulation after 48 h of starvation, we treated VSMC with EGF, FGF, and TGF-β, in addition to PDGF, and performed immunoblot analyses to examine the expression of α7-integrin. α7-Integrin protein levels in control VSMC were more variable than the higher levels observed consistently in PDGF-treated cells. Among the growth factors examined, only VSMC treated with PDGF displayed increased expression of α7-integrin consistently. Cells treated with TGF-β displayed a trend of decreasing α7-integrin levels, but this trend was not statistically significant. Notably, increased α7-integrin expression by PDGF is not due to the alteration in the differentiation status of VSMC, because no significant difference was observed in the expres-
sion of α7-actin among the various growth factors used. Overall, these results support the observation that increased α7-integrin expression is specific to PDGF.

In addition to our findings with PDGF, others have demonstrated altered expression of α7-integrin subunit by growth factors. Rosbottom et al. (33) demonstrated induction of α7-integrin subunit by TGF-β in differentiated mucosal mast cells. Also, in a porcine coronary artery occlusion model, threefold less expression of α7-integrin subunit was observed in control animals compared with animals transfected with a secretory form of FGF, as determined by microarray analysis (Forough R, unpublished observation). The differences in these various models may be attributed to differences in cell type responses to the growth factors.

Treatment with either actinomycin D or cycloheximide attenuated PDGF-induced increases of α7-integrin subunit mRNA levels. This observation suggests that PDGF modulates α7-integrin subunit expression at the transcriptional level and may depend on de novo protein synthesis. Notably, cell surface levels of α7-integrin subunit determined by FACS analysis showed significant increases at an earlier time point (12 h) than was seen with the mRNA levels. This observation is consistent with the findings that PDGF modulates the membrane mobility and trafficking of integrins (1, 32). Thus the steady-state cell surface levels of integrins may be modulated independently of increases in new mRNA and protein synthesis.

To begin to understand the signal transduction pathways by which PDGF modulates the expression of α7-integrin subunit, we investigated the contribution of various MAP kinases in α7-integrin subunit expression by using MAPK inhibitors. Our results indicate that all three MAP kinases (ERK, p38, and JNK) contribute to the regulation of α7-integrin subunit mRNA levels, because PDGF-increased α7 mRNA levels were attenuated by all three MAPK inhibitors (PD-98059, SB-203580, and SP-600125). However, whereas PD-98059 and SP-600125 significantly reduced the PDGF-increased α7-integrin subunit surface protein levels, SB-203580, a p38 kinase inhibitor, displayed no inhibitory effect. Thus our data imply that all three MAP kinases play some role in PDGF-induced changes in α7-integrin subunit mRNA levels; however, they may differentially alter cellular functions by regulating cell surface levels such as endocytic recycling of membrane proteins. This concept is supported by the observation that p38, but neither ERK nor JNK, is involved in the endocytic recycling of membrane proteins. p38 has been shown to modulate endocytic trafficking via the formation of GDI-Rab5 complex, a process that can be inhibited by SB-203580 (9). In addition, PDGF has been shown to promote Rab4-dependent recycling of αβ3-integrin from the early endosome back to the cell surface (32). Although it is beyond the scope of the present study, the effect of PDGF on α7-integrin subunit recycling and endocytosis cannot be excluded at this time.
The effect of PDGF on VSMC adherence and migration on ECM proteins, such as fibronectin and collagen, has been well studied (6, 21), whereas the effect of PDGF on VSMC adhesion to and migration on laminin has received limited attention. Because our results demonstrate increased expression of α7-integrin subunit, a laminin receptor in VSMC, we further identified potential functional alterations of VSMC on laminin. Thus, in the current studies, we examined the effect of PDGF on VSMC adhesion to and migration on laminin-1. We found that PDGF augments VSMC adhesion to laminin-1 and that this increase is mediated at least in part by α7-integrin subunit, because a function-blocking anti-α7-integrin antibody partially prevents the adhesion to laminin-1. Incubation with mouse IgG isotype control displayed no difference in adhesion to laminin-1, indicating that the blockade by α7 antibody is specific to the α7-integrin subunit. The incomplete inhibition of adhesion to laminin-1 by anti-α7-integrin antibody suggests the presence and potential involvement of other laminin receptors, such as α1-integrin subunit or the dystrophin glycoprotein complex (8, 27). Incubation with anti-α3-integrin antibody alone had no effect on adhesion to laminin-1. When PDGF-treated VSMC were incubated with the combination of anti-αβ7- and anti-α1-integrin antibodies, increased VSMC adhesion to laminin-1 was repressed to the basal level. This additive effect of antibodies suggests that collaboration between integrins may play a role in VSMC adhesion to laminin-1. In our studies, we used laminin-1 as the substrate, because αβ7 has been shown previously to mediate VSMC adhesion and migration on laminin-1 (42, 43). Others have shown that αβ7-integrin modulates adhesion of trophoblasts (23), MCF-7 carcinoma cells (38), and skeletal myoblasts (12) in a laminin isoform-specific manner.

The choice to compare the effect of α7-integrin with that of α1-integrin rather than with other laminin-binding integrins was based on our previous studies showing that α1-integrin, another laminin-binding integrin, is not present in VSMC under the culture conditions used (Wilson E, unpublished observation), and that α6-integrin, another laminin-binding integrin, is not present in VSMC (31). We also examined the effect of PDGF on α7-integrin expression by performing FACS analysis, and no significant difference was observed (data not shown).

Previously, PDGF was shown to increase the expression of other integrins such as α5-integrin (16, 19). Thus, to examine the possibility that increased adhesion of PDGF-treated VSMC to laminin-1 results from increased α5-integrin expression, we performed alternative adhesion assays with wells coated with anti-α5-, anti-αβ5-, and anti-β1-integrin antibodies. Both control and PDGF-treated cells adhered to anti-α7-integrin antibody-coated wells more effectively; no significant cell adherence was observed with the wells coated with anti-α5-integrin antibody. We also determined the expression of other integrins in control and PDGF-treated cells. Of the integrins tested (α1, α5, α6, α7, β1, and β3), only α7 and β3 showed increased expression. Minimal expression of α5-integrin level was detected under basal conditions, and PDGF has no effect on its expression (data not shown).

To investigate the extent to which increased α7-integrin subunit levels cause migratory changes of VSMC on laminin-1, we performed the migration assays using the Boyden chamber. Prolonged incubation (24 h) with PDGF did not alter serum-induced migration of VSMC on laminin-1, and preincubation with anti-α7-integrin antibody did not alter the migration of control or PDGF-treated VSMC in our study. In this respect, our results appear to contradict the observations of Yao et al. (42). Such a discrepancy may be explained on the basis of the following difference in the experimental procedures. 1) Cells were treated differently before the migration assay was performed. In our studies, VSMC were quiesced with 0.5% FBS M199 for 48 h and then treated with PDGF for 24 h before the migration assay; whereas in the study by Yao et al., cells were serum starved overnight just before the assay. 2) The choice of chemoattractant was different. We used 10% FBS M199 instead of PDGF as the chemoattractant in the lower chamber in our studies. In addition, a different source of VSMC was used in the study by Yao et al. However, our current study is consistent with the study by Ziober et al. (44), in which overexpression of α7-integrin subunit in the highly metastatic M2 cells caused the cells to be less migratory.

Studies from other groups have shown that PDGF stimulation increases expression of laminin-5 in VSMC and increases migration of VSMC on laminin-5 (22). There are several differences between these studies and our current study that may explain the discrepancy in PDGF-stimulated migration on laminin. For example, we treated the cells with PDGF 24 h before the start of the migration assay, and we used laminin-1 as a substrate, not laminin-5. Thus there may be differences in the time course of activation of migration for PDGF and differences in the specific isoform of laminin on which the cells migrate. Similarly, Crawley et al. (12) showed that anti-αβ7-integrin antibody blocked migration of skeletal myoblasts on laminin-1 but suggested that the interaction with laminin-2/4 was stronger. In addition, Yao et al. (43) previously showed that α7-integrin subunit does not interact with laminin-5. Overall, these studies suggest that cellular context and specific treatments are critical in determining the migration on laminin.

Localization of α7-integrin subunit in PDGF-treated and control VSMC was assessed using immunofluorescence imaging techniques. α7-Integrin subunit displays a punctate staining pattern in control cells, whereas in PDGF-treated VSMC, a more intense staining of α7-integrin subunit is shown and displays a stress fiber-like pattern. This result further confirms our findings with FACS analysis that PDGF augments the expression of α7-integrin subunit. The stress fiber-like staining pattern of α7-integrin subunit is consistent with the staining patterns observed in skeletal myoblast (24) and suggests that the increased expression of α7-integrin subunit is spatially associated with F-actin distribution. PDGF treatment also altered the staining of F-actin, which is consistent with previous observations showing that PDGF induces the reorganization of actin (28).

The current understanding of α7-integrin subunit in cardiovascular biology is limited. A previous study by our group (10) demonstrated that α7-integrin subunit was the only integrin with substantial increases in a chemical model of atherosclerosis. A recent study by Flintoff-Dye et al. (15) demonstrated cerebrovascular defects in embryonic mice lacking α7-integrin, which implicated compromised vascular integrity. In this study, PDGF enhanced the expression of α7-integrin and consequently increased VSMC adhesion mediated by α7-integrin.

In summary, our studies demonstrate for the first time that PDGF modulates α7-integrin subunit expression in VSMC, as
well as the contribution of MAP kinases (p38, ERK, and JNK) in regulating α7-integrin subunit expression. In addition, PDGF enhances α7-integrin subunit-mediated VSMC adhesion to laminin. The results of these studies and others suggest that the interaction between PDGF and α7-integrin may be important in vasculature development and during the remodeling process.

ACKNOWLEDGMENTS

We thank Jane Miller for technical support with flow cytometry, Nandina Para for technical support with the MAPK inhibitor study, Jeffrey Bray for excellent technical assistance with immunoblot analysis, and Stephanie Hilliard for editorial assistance. We thank Jan Patterson and Lisa Auckland for general help with this study.

Present addresses: J.-T. Chao, Department of Medical Pharmacology and Physiology, University of Missouri, Columbia, Columbia, MO 65212; G. A. Meininger, Dalton Cardiovascular Research Center, University of Missouri, Columbia, MO 65212.

GRANTS

This work was supported by National Institutes of Health Grants HL-62863 (to G. A. Meininger, K. S. Ramos, and E. Wilson), HL-62539 (to K. S. Ramos and E. Wilson), HL-58690 (to G. A. Meininger), and AG-14632 (to S. J. Kaufman); the Muscular Dystrophy Association (to S. J. Kaufman and E. Wilson); and Center for Environmental and Rural Health, Texas A&M University (to G. A. Meininger, K. S. Ramos, and E. Wilson), HL-62539 (to K. S. Ramos and E. Wilson); and Center for Environmental and Rural Health, Texas A&M University.

REFERENCES


