Original Contributions

Regulation of Platelet-Derived Growth Factor Ligand and Receptor Gene Expression by α-Thrombin in Vascular Smooth Muscle Cells

Hiroshi Okazaki, Mark W. Majesky, Laurence A. Harker, and Stephen M. Schwartz

Since the expression of genes for platelet-derived growth factor (PDGF)-A and PDGF β-receptor are reciprocally regulated in vascular wall cells after balloon injury, we have investigated the ability of specific vasoactive molecules or growth factors to reproduce the injury pattern of gene expression in cultured rat smooth muscle cells (SMCs) and assessed the effect of inactivating α-thrombin on injury-induced expression of PDGF-A mRNA by vascular wall cells in vivo. The molecules investigated, to which vascular SMCs may be locally exposed after mechanical injury, included vasoactive factors (α- and β-adrenergic agonists, serotonin, histamine, angiotensin II, and endothelin) and growth factors (PDGF-AA, PDGF-BB, basic fibroblast growth factor, insulin-like growth factor, epidermal growth factor, and α-thrombin). In cultured rat SMCs, only α-thrombin (0.1–100 nM), among these compounds, produced the pattern of transiently increased PDGF-A and decreased PDGF β-receptor mRNA. PDGF-B chain mRNA levels remained undetectable in these cultured SMCs. The dependence of these changes in gene expression on the proteolytic activity of α-thrombin was shown by the interruption of altered gene expression or DNA synthesis after incubating the cultured SMCs with covalently inactivated α-thrombin using D-Phε-Pro-Arg chloromethyl ketone, a synthetic direct active-site irreversible inhibitor of α-thrombin. Continuous intravenous infusion of this synthetic antithrombin into baboons for 6 hours (100 nmol/kg per minute maintaining constant plasma levels of 3.0±0.5 μg/ml) after inducing balloon-catheter arterial injury also prevented the threefold increase in expression of PDGF-A mRNA characteristically exhibited by untreated mechanically injured vessels. We conclude that α-thrombin initiates an injury pattern of PDGF ligand and receptor gene expression both in vitro and in vivo. The importance of α-thrombin in the development of vascular lesion formation induced by mechanical injury and the possible usefulness of interrupting α-thrombin activity in the prevention of restenosis remain to be determined. (Circulation Research 1992;71:1285–1293)

KEY WORDS • α-thrombin • platelet-derived growth factor • balloon catheter injury • smooth muscle cell proliferation

Injury to the vessel wall by passage of a balloon catheter induces smooth muscle cell (SMC) proliferation.1-4 The response-to-injury hypothesis proposes that platelets adhere to the vessel wall and release mitogens.5 Experiments using detailed cell kinetic analysis and antiplatelet antibodies, however, have not shown that this pathway is responsible for SMC replication in vivo.6-9 Moreover, proliferation persists for several weeks after the platelet response has abated. These data, as well as evidence that mitogens are produced by vessel wall cells themselves,10-13 suggest that those mitogens produced by vessel wall cells may control SMC replication.

Previously, we have shown that the expression of platelet-derived growth factor (PDGF) ligand and PDGF receptor genes are modulated by the arterial wall after balloon catheter injury.11 This injury pattern included substantial increases in PDGF-A gene expression and reciprocal decreases in PDGF β-receptor gene expression.11 These findings suggest that these alterations in gene expression may constitute an injury-induced vascular repair response that could control SMC proliferation.

Because the mechanisms giving rise to changes in gene expression of PDGF ligand and PDGF receptor within the vessel wall have not been defined, we evaluated the effects of specific molecules that might be generated at sites of denuding vascular injury on the expression of PDGF ligand and PDGF receptor genes in cultured adult rat arterial SMCs and balloon catheter–injured arteries in vivo. The molecules investigated included α- and β-adrenergic agonists, serotonin, histamine, angiotensin II, endothelin, PDGF-AA, PDGF-BB, basic fibroblast growth factor (bFGF), insulin-like growth factor (IGF)-1, epidermal growth factor (EGF),
and \( \alpha \)-thrombin. The only candidate mediator reproducing the injury pattern of PDGF ligand and PDGF receptor mRNA expression in cultured SMCs was \( \alpha \)-thrombin. To examine the in vivo relevance of these cell culture studies, we investigated the effects of continuously infused \( \beta \)-Phe-Pro-Arg chloromethyl ketone (D-FPRCH\(_2\)Cl), a potent direct irreversible antithrombin, on gene expression in nonhuman primates undergoing balloon catheter arterial injury. These studies demonstrated a decrease in PDGF-A chain expression, implying that thrombin or a similar protease mediates the elevation of the PDGF-A chain in vivo as well.

**Materials and Methods**

**Cell Culture**

Thoracic aortas from 3-month-old (adult) male Wistar rats were removed and stripped of endothelium and adventitia, and medial SMCs were obtained by collagenase and elastase digestion.\(^{14}\) Rat aortic SMCs were routinely grown in Waymouth’s medium supplemented with 10% adult bovine serum (Hyclone Laboratories, Logan, Utah) and were used between the fourth and twelfth passage. After reaching confluence, the medium was replaced with serum-free Waymouth’s medium, and agents were added 2 days later.

**RNA Isolation and Blot Hybridization**

Total cellular RNA was prepared by acid guanidium thiocyanate extraction as previously described.\(^{15}\) RNA samples were electrophoresed through 1.2% agarose gels submerged in 2.2 M formaldehyde/10 mM sodium phosphate (pH 7.2), transferred to a nylon membrane (Zeta probe, Bio-Rad Laboratories, Richmond, Calif.) in 3 M NaCl/0.3 M sodium citrate, and baked at 80°C for 2 hours. Membranes were hybridized in 50% (vol/vol) formamide/0.25 M sodium phosphate (pH 7.2)/7% sodium dodecyl sulfate/0.25 M NaCl/1 mM EDTA/10% (wt/vol) polyethylene glycol\(^{16}\) containing 100 \( \mu \)g salmon sperm DNA/ml at 42°C for 24 hours with DNA probes labeled with \([\text{\textsuperscript{32}P}]\)dCTP by random oligonucleotide priming (Amersham Corp., Arlington Heights, Ill.). Nylon membranes were exposed briefly to shortwave ultraviolet light to cross-link RNA to the membrane. Blots were washed twice at 60°C in 0.03 M NaCl/0.003 M sodium citrate (pH 7.0)/0.1% sodium dodecyl sulfate for 15 minutes each and then exposed to Kodak X-AR5 film at \(-70^\circ\text{C}\).

**DNA Probes**

DNA probes used for RNA blot hybridization were as follows: PDGF-A, a 1.3-kb EcoRI human cDNA fragment released from pD1\(^{17}\); PDGF-B, a 2.1-kb Sac I–Sac II human cDNA fragment from pSM-1\(^{18}\); PDGF \( \beta \)-receptor, a 4.7-kb EcoRI–Xba I human cDNA fragment from pHPDGFR\(^{19}\); PDGF \( \alpha \)-receptor, a 6.4-kb EcoRI rat cDNA from p802E/B5\(^{20}\); and glyceraldehyde-3-phosphate dehydrogenase (GAPDH), a 1.2-kb Pst I human cDNA fragment from pHc GAP.\(^{21}\)

**Thymidine Incorporation**

The cells were plated at 2.4\( \times \)10\(^4\) cells per 24-well culture well in 1 ml Waymouth’s medium with 5% adult bovine serum. Three days after plating, media were replaced with serum-free Waymouth’s medium. When the cells had become quiescent (3–4 days), the test samples were added directly to the wells and incubated for 24 hours at 37°C. After 24 hours, 1 \( \mu \)Ci/ml [\( \text{\textsuperscript{3}H} \)]thymidine (6.7 Ci/mol) was added to each well for 2 hours. The trichloroacetic acid (TCA)–precipitable material was harvested by aspirating the media, washing the cell layer twice with ice-cold 5% TCA, and solubilizing the TCA-insoluble material in 0.8 ml of 0.25 NaOH with gentle mixing for 10–15 minutes. The solubilized material (0.6 ml) was added to 5 ml Aquasol-2 (New England Nuclear, Dupont Co., Claremont, Calif.), and radioactivity was determined by liquid scintillation counting.

**Baboon Arterial Injury Model**

The in vivo effects of inhibiting \( \alpha \)-thrombin on the increased expression of PDGF-A mRNA induced by mechanical arterial injury was studied in six juvenile male baboons (Papio anubis) weighing 8–12 kg, previously observed to be disease free for 3 months. All procedures were performed under sterile conditions and were approved by the institutional animal use and care committee and conducted in accordance with federal guidelines.

To produce acute arterial injury, balloon catheter denuding angioplasty of the left brachial arteries was performed according to a standard method\(^{22}\) on anesthetized animals, using ketamine (20 mg/kg i.m.) as the preanesthetic and halothane (1%) as the inhalation agent. After an incision performed over the medial aspect of the forearm, a side branch of the brachial artery was isolated and controlled using vessel loops. A 3F Fogarty balloon catheter was passed through the branch to a distance of 10 cm, inflated to a diameter of approximately 4 mm by filling with sterile saline, and withdrawn the length of the vessel using a gentle twisting motion. A moderate, but not strong, resistance to the passage of the balloon was achieved in all cases. To ensure complete deendothelialization, this procedure was repeated three times with no instance of vessel rupture. After balloon inflation, the catheter was withdrawn, the access vessel was ligated, and the incision site was closed. In each animal, only the left brachial artery was balloon-inflated, with the contralateral artery serving as an uninjured control vessel.

In half of the animals, D-FPRCH\(_2\)Cl was administered by continuous intravenous infusion (100 nmol/kg per minute) beginning before the balloon catheter injury and extending throughout the 6-hour period until the brachial arteries were harvested. At that dose, this synthetic irreversible antithrombin potently inhibits thrombin activity and abolishes thrombus formation at sites of mechanical arterial injury.\(^{23,24}\)

Six hours after arterial balloon inflation, the animals were anesthetized, both brachial arteries were harvested, and periadventitial tissues were stripped off in phosphate-buffered saline at 4°C. Endothelium was removed by gently scraping the luminal surface with the edge of a Teflon card. Arteries were then snap-frozen in liquid nitrogen for subsequent RNA isolation. Frozen arterial tissue was ground to a fine powder under liquid nitrogen, and total cellular RNA was prepared. We performed the same in vivo experiment twice.
Materials

Purified human α-thrombin and D-Phc-Pro-Arg-CH₂Cl-α-thrombin (D-FPRCH₂Cl-α-thrombin) were generously provided by Dr. John Fenton II, Albany, N.Y. The specific activity of α-thrombin was 2,875.12 NIH units/mg. The specific activity of the D-FPRCH₂Cl-α-thrombin was 0.067 NIH unit/mg. The following drugs were obtained from Sigma Chemical Co., St. Louis, Mo.: L-phenylephrine HCl, propranolol HCl, isoproterenol HCl, histamine di-HCl, norepinephrine bitartrate, serotonin creatinine sulfate, and angiotensin II. Endothelin was purchased from Cambridge Research Biochemicals, Valley Stream, N.Y. The following growth factors were obtained from Upstate Biotechnology, Inc.: PDGF-AA, PDGF-BB, bFGF, IGF-1, and EGF.

Results

Effects of α-Thrombin and Other Vasoactive Factors on PDGF-A Chain mRNA Levels in Cultured Adult Rat SMCs

We reported previously that arterial injury evokes large transient increases in levels of PDGF-A chain mRNA in balloon-injured carotid arteries. The present in vitro studies were designed to determine whether PDGF-A chain mRNA expression in cultured rat adult SMCs is regulated by specific molecules to which vascular SMCs may be exposed after injury. Quiescent SMCs were incubated with vasoactive factors, and PDGF-A chain mRNA levels were measured by Northern blot analysis. Both α-thrombin and serotonin strikingly increased PDGF-A chain mRNA 6 hours after initiating incubation (Figure 1). Norepinephrine, phenylephrine, angiotensin II, and PDGF-BB produced small increases in PDGF-A chain mRNA levels. Histamine, isoproterenol, endothelin, PDGF-AA, bFGF, IGF-1, and EGF caused no significant changes in PDGF-A chain mRNA levels (Figure 1). We have also examined the effect of tissue plasminogen activator as one possible other plasma protease that might be produced in the artery after balloon injury. Tissue plasminogen activator had no effect on PDGF-A chain mRNA levels in rat cultured SMCs (data not shown).
Effects of α-Thrombin and Serotonin on PDGF Receptor mRNA Levels in Cultured Adult Rat SMCs

We previously observed that levels of PDGF β-receptor mRNA are decreased 6 hours after balloon injury in carotid arteries in vivo.11 Therefore, we determined whether either α-thrombin or serotonin reduced PDGF β-receptor mRNA levels of cultured rat vascular SMCs. Notably, α-thrombin markedly decreased PDGF β-receptor mRNA levels in rat adult SMCs 6 hours after initiating exposure, whereas serotonin failed to affect the expression of PDGF β-receptor mRNA levels (Figure 2). Thus, among the molecules tested, only α-thrombin reproduced both the upregulation of PDGF-A mRNA levels and the downregulation of PDGF β-receptor mRNA levels 6 hours after incubation with adult cultured vascular SMCs.

α-Thrombin Stimulates Transient Increase in PDGF-A Chain mRNA and Rapid Loss of PDGF Receptor mRNA

To examine the time course for thrombin's regulation of PDGF-A and PDGF receptor gene expression, total cellular RNA was isolated and measured by RNA transfer blot analysis at various times after initiating the incubation of cells with α-thrombin.

PDGF-A

A family of PDGF-A transcripts (2.9, 2.3, 1.7 kb) was present at very low levels in confluent cultured rat adult SMCs (Figure 3). α-Thrombin increased the expression of 2.9- and 2.3-kb transcripts for PDGF-A mRNA. Peak levels were achieved 4 hours after incubating α-thrombin and cells, and the values remained elevated for 12 hours. By 24 hours, these PDGF-A transcript levels returned to the basal level. The level of 1.7-kb transcripts for PDGF-A did not show useful changes (Figure 3). PDGF-B chain mRNA levels were undetectable in these cultured rat adult SMCs (data not shown).

PDGF β-Receptor

Easily identifiable levels of a single 5.7-kb transcript for PDGF β-receptor were found in confluent cultured rat adult SMCs (Figure 3). Rapid loss of PDGF β-receptor mRNA occurred in the first 6 hours after exposure to α-thrombin (Figure 3). PDGF β-receptor mRNA levels were reduced approximately 30% at 4 hours, and further reductions in PDGF receptor mRNA were found at 6 hours, totaling an approximate 70% loss in these cells.
of receptor transcripts compared with the basal level. By 48 hours, reduced PDGF receptor transcript levels gradually returned to the basal values.

**PDGF α-Receptor**

A single 6.5-kb transcript for PDGF α-receptor was identified in confluent rat cultured SMCs (Figure 3). A significant reduction in PDGF α-receptor mRNA levels was found in the first 6 hours after exposure to α-thrombin (Figure 3). PDGF α-receptor mRNA levels were maximally reduced at 6 hours and gradually returned to the basal levels by 48 hours.

**Dose-Dependent Changes in α-Thrombin-Induced PDGF-A and PDGF β-Receptor Gene Expression**

α-Thrombin stimulated PDGF-A gene expression in cultured rat SMCs in a dose-dependent manner (Figure 4). The levels of 2.3-kb PDGF-A transcript were significantly increased 4 hours after exposure to 0.1 nM α-thrombin. Increasing doses of α-thrombin from 1.0 to 100 nM produced concordant increases in 2.3-kb PDGF-A mRNA levels. The reduction in PDGF β-receptor gene expression in rat SMCs by α-thrombin was also dose dependent after 4 hours of incubation with α-thrombin at concentrations of 1.0 and 100 nM (Figure 4).

**Dependence of PDGF-A and PDGF β-Receptor Gene Expression on α-Thrombin’s Catalytic Activity**

To determine whether PDGF-A and PDGF β-receptor mRNA regulation by α-thrombin is mediated through its proteolytic activity, α-thrombin’s catalytic activity was covalently inactivated with D-FPRCH2Cl, a potent synthetic antithrombin III–independent antithrombin. D-FPRCH2Cl-α-thrombin was incubated with rat adult SMCs, and PDGF-A chain and PDGF β-receptor mRNA expression was assessed. D-FPRCH2Cl-α-thrombin failed to increase the expression of PDGF A gene (Figure 5) or to reduce the expression of PDGF β-receptor gene (Figure 5). Instead, D-FPRCH2Cl-α-thrombin produced a small increase in the level of PDGF β-receptor mRNA (Figure 5). Thus, both PDGF-A gene upregulation and PDGF β-receptor gene downregulation in cultured SMCs by α-thrombin are dependent on the presence of enzymatically active α-thrombin.

**Comparison of Mitogenic Effects Produced by α-Thrombin and D-FPRCH2Cl-α-Thrombin in Cultured Rat Adult SMCs**

To compare the growth-promoting effects of α-thrombin and enzymatically inactive D-FPRCH2Cl-α-thrombin, adult rat SMCs were growth-arrested for 3–4 days in serum-free Waymouth’s medium. [3H]Thymidine in-

**Figure 4.** Graph showing that α-thrombin-induced changes in platelet-derived growth factor (PDGF)-A and PDGF β-receptor gene expression are dose dependent. Quiescent confluent smooth muscle cells were exposed to the indicated concentrations of α-thrombin for 4 hours. Total RNA (12 μg) per lane was subjected to Northern analysis for PDGF-A and PDGF β-receptor mRNA. Autoradiographs of RNA transfer blots were scanned with a laser densitometer, and relative signal intensities were plotted.

**Figure 5.** Enzymatic activity of α-thrombin is involved in the regulation of platelet-derived growth factor (PDGF)-A and PDGF β-receptor (PDGF-Rβ) gene expression. Quiescent confluent rat vascular smooth muscle cells were treated with 10 nM D-Phe-Pro-Arg-CH2Cl-α-thrombin for the indicated times. Total cellular RNA (12 μg) per lane was examined. The same blot was hybridized sequentially with PDGF-A, PDGF-Rβ, and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) probes.
corporation was then determined after exposure to α-thrombin or D-FPRCH2Cl-α-thrombin for 24 hours. As shown in Figure 6, α-thrombin stimulated [3H]thymidine incorporation into DNA in a dose-dependent manner. The ED₅₀ for α-thrombin was 1.0 nM, and a maximal 10-fold increase was observed at a concentration of 10 nM. In contrast, D-FPRCH2Cl-α-thrombin had no significant effect on [3H]thymidine incorporation in rat adult SMCs. The small increase in DNA synthesis observed with 100 nM D-FPRCH2Cl-α-thrombin may be attributable to minimal residual contamination with α-thrombin, since levels as low as 0.1 nM α-thrombin caused a small increase in [3H]thymidine incorporation. These findings indicate that stimulated [3H]thymidine incorporation into cultured SMCs by α-thrombin requires proteolytically active α-thrombin.

Effects of D-FPRCH2Cl on PDGF-A Gene Expression Induced In Vivo by Balloon Catheter Injury

Although the culture studies used rat SMCs, we chose to perform the in vivo studies in baboons. This was done because of a lack of data on the efficacy of antithrombin agents in rats and because the platelet, coagulation, fibrinolytic, and inhibitor molecular interactions in baboons closely resemble those in humans. The dose–response relation, regimen, and safety of continuous intravenous infusions of D-FPRCH2Cl have been well established in baboons, and the response of baboon vessels to balloon injury is very similar to that found in humans.

A family of PDGF-A transcripts (2.9, 2.3, and 1.7 kb) was present at low levels in uninjured control baboon brachial arteries (Figure 7), similar to the results for control rat carotid arteries. Balloon catheter injury induced a threefold increase in PDGF-A gene expression (normalized by GAPDH mRNA levels) in balloon-injured left brachial arteries 6 hours after injury compared with the contralateral unmanipulated arteries (Figure 7). However, there was no detectable loss of PDGF β-receptor mRNA levels after balloon injury in these specimens (data not shown). The injury-induced increase in PDGF-A transcript levels was reduced by

![Figure 6. Graph showing the effect of α-thrombin and D-Phe-Pro-Arg-CH2Cl-α-thrombin (FPRCH2Cl-α-thrombin) on DNA synthesis in rat cultured vascular smooth muscle cells. Confluent quiescent rat smooth muscle cells were exposed to the indicated concentrations of α-thrombin and FPRCH2Cl-α-thrombin for 24 hours and pulse-labeled with [3H]thymidine for 2 hours to determine relative rates of DNA synthesis.](image)

![Figure 7. Effects of D-Phe-Pro-Arg-CH2Cl (D-FPRCH2Cl) on platelet-derived growth factor (PDGF)-A gene expression by balloon injury in the baboon model. Six hours after balloon injury, the animals were anesthetized, and both brachial arteries were harvested and stripped of periadventitial tissues and endothelium. Arteries were then snap-frozen in liquid nitrogen for subsequent RNA isolation. Total RNA (12 µg) per lane was subjected to Northern analysis for PDGF-A and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA. The same in vivo experiment (Exp) was performed twice.](image)
the continuous intravenous infusion of D-FPRCH2Cl for 6 hours (Figure 7). We performed the same in vivo experiment twice and achieved almost the same result (Figure 7).

Discussion

This study shows that catalytically active α-thrombin induces dose-dependent increases in PDGF-A gene expression and proliferation together with reciprocal decreases in PDGF β-receptor gene expression in cultured vascular SMCs. In addition, infusion of an inhibitor of α-thrombin’s proteolytic activity interrupts injury-induced augmentation of PDGF-A mRNA expression in vascular wall cells in vivo. These findings suggest that α-thrombin may be an important mediator of the pattern of gene expression of an endogenous growth factor after injury to the vessel wall and suggest that α-thrombin may be a useful target for prevention of stenosing lesion formation after mechanical vascular damage.

We reported previously that RNA levels of the PDGF ligand and receptor genes are modulated in the vessel wall during its response to balloon catheter injury11; the response pattern includes elevation of PDGF-A gene expression and depression of PDGF β-receptor gene expression. In the present study, we compared the in vitro effects of molecules that may be generated at the site of vascular injury and are therefore candidate mediators of the endogenous pattern of regulated gene expression in the injured vessel wall. Among the molecules tested on cultured rat SMCs, only α-thrombin reproduced the injury pattern of PDGF-A chain and PDGF β-receptor gene expression. Although the similarities between the pattern of gene expression after thrombin is given in vitro and the pattern seen after balloon angioplasty in vivo could be coincidental, our results suggest that the generation of α-thrombin in the thrombus accumulating at the site of vascular injury could play a critical role in controlling vascular lesion formation induced by mechanical damage.

α-Thrombin was selected for study because this protease exhibits mitogenic effects on rat cultured vascular SMCs.27 The presence of α-thrombin-like activity has been observed on the damaged vascular surface studied ex vivo after balloon catheter injury.28 α-Thrombin was also of interest because it stimulates expression of PDGF-A chain in cultured endothelial cells and mesangial cells.29,30 Norepinephrine, phenylephrine, and isoproterenol were tested because, among sympathomimetic agents, only α-adrenergic agonists induce PDGF-A gene expression in the rat thoracic aorta.31 Angiotensin II was included for study because it also stimulates PDGF-A gene expression in rat aortic SMCs in vitro24 and because angiotensin II infusions stimulate [3H]thymidine incorporation into aortic tissue of Sprague-Dawley rats.32 Endothelin was included for study because it stimulates c-fos and c-myc expression and increases the proliferation of vascular SMCs in vitro.33,34 Serotonin and histamine were studied because they are secreted from platelets during activation and have been reported to exhibit mitogenic effects on cultured vascular SMCs.35,36 PDGF was tested because it is a major regulator of protein synthesis37 and growth in cultured arterial SMCs38 and because SMCs synthesize and secrete PDGF-like molecules under certain conditions.40–42 bFGF was investigated because vascular SMCs produce bFGF and it stimulates the proliferation of SMCs in culture.43 Moreover, recent studies using infused bFGF or infused antibodies to bFGF implicate bFGF as a critical endogenous mitogen responsible for the initial wave of smooth muscle replication after balloon injury.10 IGF-1 and EGF were examined because they stimulate the proliferation of vascular SMCs.44,45 PDGF, bFGF, IGF-1, and EGF also stimulate PDGF-A gene expression in mesangial cells in culture.46 Agents were tested at concentrations based on previously reported experience for demonstrating vasoactive effects or mitogenic effects of these agents.

α-Thrombin and serotonin induced greater PDGF-A chain expression than did the other agents studied. Norepinephrine, phenylephrine, angiotensin II, and PDGF-BB produced minimal increases in PDGF-A mRNA levels. No changes in PDGF-A mRNA levels were induced by endothelin, isoproterenol, histamine, PDGF-AA, bFGF, IGF-1, or EGF. Although phenylephrine, norepinephrine, and angiotensin II have been reported to increase PDGF-A gene expression in the rat aorta, endothelin and isoproterenol failed to induce detectable PDGF-A gene expression in vivo.33 Thus, our in vitro findings are in accord with the available data in vivo.

Changes in the mRNA levels for PDGF β-receptor gene were also investigated in the cultured adult SMCs. Of the agents studied, only α-thrombin decreased the levels of PDGF β-receptor mRNA. Additionally, the time course of PDGF ligand and receptor gene expression after exposure to α-thrombin demonstrated peak changes by 4 and 6 hours, respectively, and normalization by 48 hours. Thus, α-thrombin, unique among the panel of potential agonists studied in culture, reproduced the regulated injury pattern of PDGF-A chain mRNA and PDGF β-receptor mRNA developing in injured rat arterial SMCs in vivo. The hypothesis that α-thrombin controls the change in PDGF-A mRNA abundance in vivo is consistent with the pattern of thrombinlike activity after balloon catheter injury.28 Hatton et al.28 showed that deendothelialized rabbit aorta exhibited thrombinlike activity that was maximal approximately 50 minutes after balloon injury and was detectable for up to 10 days after injury. In our experiment, arterial injury increases PDGF-A gene expression at 6 hours and is identifiably increased 1 week after injury.11 Thus, the temporal relations are concordant with the possibility that α-thrombin activity in the vessel wall may mediate the injury pattern of PDGF-A and PDGF β-receptor gene expression.

Increase in abundance of PDGF-A mRNA by α-thrombin depended on the proteolytic activity of α-thrombin. Proteolytically inactive α-thrombin, D-FPRCH2Cl-α-thrombin, showed very low or undetectable levels of increase in PDGF-A gene expression. α-Thrombin stimulation of [3H]thymidine incorporation was also mediated through proteolytic activity. D-FPRCH2Cl-α-thrombin had no mitogenic effect, and γ-thrombin, catalytically active with obstructed anion-binding site required for clotting activity, showed the same extent of [3H]thymidine incorporation as α-thrombin (data not shown). We also found that α-thrombin regulates PDGF β-receptor gene expression via proteolytic activity, since we saw no decrease in PDGF β-re-
ceptor mRNA when α-thrombin was inactivated with D-FPRCH2Cl. Indeed, there was a small increase in the levels of PDGF β-receptor mRNA. These observations contrast with the report that both α-thrombin and DIP-α-thrombin stimulated PDGF-B gene expression without proteolytic activity in microvascular endothelial cells and with a recent report claiming that nonenzymatic α-thrombin is also mitogenic in bovine aortic medial cells. It is possible that different pathways are involved in the regulation of PDGF-A gene expression by α-thrombin in these two cell types or in the different species.

The decrease of PDGF β-receptor gene expression probably does not represent an autocrine downregulation by PDGF secreted by the stimulated smooth muscle. Although the PDGF α-receptor binds the three isoforms with high affinity and the PDGF β-receptor binds PDGF-BB with high affinity and PDGF-AB with low affinity and does not bind PDGF-AA, no regulation of PDGF α- and PDGF β-receptor mRNA levels was found when either PDGF-AA or PDGF-BB was added in this culture system (data not shown). These findings imply that the decrease in PDGF α-receptor mRNA levels is also directly regulated by α-thrombin. The lack of detectable PDGF-B mRNA is consistent with our previous data and the report by Sjölund et al.

Finally, we determined whether infusion of a protease inhibitor interrupts the gene expression of PDGF-A chain after vascular injury in the nonhuman primate. The baboon was used because of its demonstrated similarities to humans with respect to the molecular interactions among vascular and blood constituents leading to acute thrombus formation and vascular lesion formation at sites of mechanical injury. Regulation of injury-induced vascular proliferative responses in the rat may be different from primates under some circumstances. In addition, the dosage regimen and safety are established in this species. Balloon injury induced elevated levels of mRNA for PDGF-A, as we had seen in the rat. However, we did not see a decrease in PDGF β-receptor gene expression, perhaps because of differences in species. The 6-hour continuous intravenous infusion into baboons of D-FPRCH2Cl, a potent irreversible inhibitor of α-thrombin, prevented the injury-induced increase in PDGF-A gene expression characteristically observed after balloon injury in untreated control baboon brachial arteries. These data indicate that α-thrombin is involved in the increase in PDGF-A gene expression after balloon injury in vivo.

In summary, we find that α-thrombin regulates levels of PDGF ligand and PDGF receptor mRNA with similar patterns in vitro and in vivo. The inhibition of PDGF-A chain mRNA expression by inactivating α-thrombin suggests that proteolytic events associated with acute thrombus formation occurring at the site of vascular injury may represent appropriate targets for therapeutic intervention in the vascular proliferative response induced by mechanical injury.

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References


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