Altered PDGF-BB–Induced p38 MAP Kinase Activation in Diabetic Vascular Smooth Muscle Cells

Roles of Protein Kinase C-δ

Hiroshi Yamaguchi, Masahiko Igarashi, Akihiko Hirata, Naoko Sugae, Hiromi Tsuchiya, Yumi Jimbu, Makoto Tominaga, Takeo Kato

Objective—We investigated the regulation of p38 mitogen-activated protein kinase (MAPK) by platelet-derived growth factor (PDGF)-BB and its biological effects in cultured normal and diabetic rat vascular smooth muscle cells (VSMCs).

Methods and Results—VSMC growth from diabetic rats was faster than that from normal rats. The expression of the PDGF β-receptor in diabetic VSMCs was significantly elevated compared with that in normal cells, and PDGF-BB–induced p38 phosphorylation in diabetic cells was more enhanced via MAPK kinase (MKK) 3/6. The level of PKC activity in diabetic cells increased more than that in normal cells with or without PDGF-BB. Although protein kinase C (PKC)-βII and PKC-δ were activated by diabetes, PDGF-BB could further enhance the level of PKC-δ alone. PDGF-BB–induced cell migration was more elevated in diabetic VSMCs, and the increase was significantly inhibited by SB-203580, rottlerin, and antisense oligodeoxynucleotides for PKC-δ. PDGF-BB–induced p38 phosphorylation also regulated cell growth, cyclooxygenase-2 levels, and arachidonic acid release, but not apoptosis. These levels were more elevated in diabetic cells, which were inhibited by SB-203580.

Conclusions—Our study established that PDGF-BB phosphorylated p38 via PKC-δ and the subsequent MKK 3/6, leading to cell growth regulation and the progression of a chronic inflammatory process in diabetic VSMCs. (Arterioscler Thromb Vasc Biol. 2004;24:2095-2101.)

Key Words: p38 □ PDGF-BB □ VSMC □ PKC □ migration

Diabetes mellitus is a most crucial risk factor for atherosclerosis.1,2 Several hypotheses, such as hyperosmolarity,3 advanced glycation end products,4 oxidant formation,5 abnormality of sorbitol and myoinositol metabolism,6 and diacylglycerol protein kinase C (PKC) activation,7 have been proposed to explain the various pathological changes induced by hyperglycemia in vasculature. Glucose and its metabolites possibly mediate their adverse effects by altering the various signal transduction pathways used by vascular cells to perform their functions and maintain cellular integrity. Some changes in vascular cells include increases in contractility, cellular proliferation, migration, and extracellular matrix production, which are abnormal in diabetes.7 We and other investigators recently identified that the activation of p38 mitogen-activated protein kinase (MAPK) could be responsible for some vascular dysfunctions observed in the diabetic state.8–11 However, it has not been determined whether hyperglycemia and its metabolites can affect other signal transduction systems and/or the cellular targets of p38 activation.

In contrast, the platelet-derived growth factor (PDGF) is recognized as a major mitogen in serum and one of the most important growth factors that promote atherogenesis, including the proliferation of vascular smooth muscle cells (VSMCs).12–15 PDGF-BB can bind all types of receptors and activate the GTPase-activating protein of Ras (Ras-GTP) and, subsequently, extracellular-regulated protein kinase (extracellular signal regulated kinase [ERK]) 1/2, leading to cellular proliferation and differentiation by stimulating various transcription factors inducing the expression of growth-responsive genes.13–15 In contrast, recent reports have shown that PDGF-BB–induced p38 may be involved in vascular remodeling.16–18 However, it is still unknown whether PDGF-BB–induced p38 activation results in diverse cellular responses, especially in a diabetic condition.

A better understanding of the precise molecular and cellular mechanisms in diabetes is necessary to evaluate the effect of PDGF-BB on p38 activation from a clinical viewpoint. Therefore, in this study, we examined the regulation of p38 by PDGF-BB and its biological effects in cultured normal and diabetic rat VSMCs.
Methods

Cell Culture
Male Sprague–Dawley rats (≈200 g) were used for this procedure. Diabetes in rats was induced by a single intravenous injection of sterile streptozotocin (STZ; 50 mg/kg body weight; Sigma) solubilized with a 20 mmol/L citrate buffer (pH 4.5). Control rats were also subjected to the same procedure without STZ.

Then, VSMCs were harvested from the aortae of rats by the media explant technique. At day 10 after explant, in a single flask, the percentage value of the number of VSMC outgrowth-positive blocks/number of total plated blocks was used as the outgrowth rate, as described previously. In addition, to clarify the difference in cell growth between normal and diabetic VSMCs, a cell growth assay was performed in each passage as described previously.

Immunoblot Analysis of Phosphorylated p38, MAPK Kinases, and PDGF Receptors
A previously described experimental procedure was used. VSMCs were pretreated with each inhibitor solubilized with DMSO for 30 minutes and incubated during the experiments, as well. Because the phosphorylation of p38 by PDGF-BB in normal rat VSMCs was already reported, the 5-ng/mL and 5-minute condition after PDGF-BB (Pepro Tech EC) stimulation was used in the following experiments.

In Situ PKC Activity Assay
PKC activity was measured by using a modified in situ method as described previously. The kinase reaction was terminated with 5% trichloroacetic acid.

Immunoblot Analysis of PKC Isoforms
The cultured VSMCs were prepared as mentioned. PKC in membra- nous and cytosolic fractions was partially purified using DEAE cellulose (Pharmacia Biotech). Levels of various types of PKC isoforms were detected by immunoblot.

Migration Assay
Cell migration was assayed by a modification of Boyden chamber method using a polycarbonate filter (Neuro Probe) with an 8.0-μm diameter pore. Migration activity was expressed as the mean number of migrated cells in 4 different fields.

Oligodeoxynucleotide Treatment
Based on a previous report, we used 2 oligodeoxynucleotides corresponding to the translation initiation regions of mRNA specific for human PKC-α. The sequences of antisense and sense ODN were 5’-AGGGGGTGCAT- GATGGA-3’ and 5’-TCGATCATGCGACCAA-3’, respectively (Sawady Technology, Tokyo, Japan).

Measurement of DNA Contents
The assay was performed as described previously. After being starved, they were preincubated with various concentrations of SB-203580 for 4 hours, and 5 ng/mL of PDGF-BB or vehicle was added to each well. After another 16-hour incubation, 1 μCi of [3H]-thymidine was added to each well and incubated for 4 hours.

In Situ Apoptosis Assay
The VSMCs were plated on an 8-well Laboratory-Tek chamber and subcultured. After being starved, the cells were preincubated with or without SB-203580 for 30 minutes, and PDGF-BB or anisomycin was added to each well and cultured for another 15 minutes. Then, a TACS in situ apoptosis detection kit using the terminal deoxynu- cleotidyld transferase (TdT)-mediated dUTP-biotin nick-end labeling (terminal deoxynucleotidyld transferase-mediated dUTP nick end-labeling [TUNEL]) method was performed.

Results
Effect of Diabetes on the Growth Properties of VSMCs
The VSMC outgrowth rate in an aortic medial explant from normal and diabetic rats at 10 days is shown in Figure 1A. The value in diabetes was significant, i.e., 3-fold higher than that in normal. In a subculture from diabetic rats, VSMCs grew faster than in one from normal rats until the fifth passage, and, in diabetes, the cell number was significantly higher than in normal after a 3-day subculture with medium...
PDGF increased by PDGF-BB. diabetic than in normal cells, and the activity was remarkably in situ PKC activity were significantly more elevated in As shown in Figure 2A, without any stimulation, the levels of PDGF-BB Stimulation Effect of PKC on Diabetes and Its Upstream Level

Figure 2. A, In situ PKC activity in normal and diabetic VSMCs. The assay was performed in duplicate (n=6, respectively). *P<0.01 versus PDGF-BB(−) in normal cells; †P<0.01 versus PDGF(−) in diabetic cells. B, Effect of PDGF-BB on the activation of PKC isoforms in normal and diabetic VSMCs. The quantification of the level of each PKC isoform from 4 separate experiments is shown in the lower panel. *P<0.05 versus PDGF-BB(−) in normal cells; †P<0.05 versus PDGF(−) in diabetic cells.

Effect of PDGF-BB on p38 Phosphorylation and Its Upstream Level

As shown in Figure 1C, without any stimulation, the level of phosphorylated p38 in diabetic VSMCs was significantly elevated to 155±9% of that in normal ones. In addition, compared with the respective control, the levels of phosphorylated p38 were remarkably increased to ~3-fold by PDGF-BB; the levels were dose-dependently inhibited by SB-203580, a p38-specific inhibitor, in normal and diabetic cells.

Next, we examined the roles of MAPK kinases (MKKs) existing in the upstream level of PDGF-BB–induced p38 phosphorylation in VSMCs. Without any stimulation, the levels of MKK 3/6 (Figure 1D) and MAP kinase 1/2 (MEK 1/2) (data not shown) phosphorylations were significantly more elevated in diabetic VSMCs than in normal cells, and these phosphorylations were remarkably increased by PDGF-BB. In contrast, differently from SB-203580, PDGF-BB–induced p38 phosphorylation was unchanged by 5 μmol/L of PD-98059, an MEK 1/2–specific inhibitor (Figure 1E).

Effect of PKC on Diabetes and PDGF-BB Stimulation

As shown in Figure 2A, without any stimulation, the levels of in situ PKC activity were significantly more elevated in diabetic than in normal cells, and the activity was remarkably increased by PDGF-BB.

Subsequently, to characterize the effects of type(s) of PKC isoforms on PDGF-BB stimulation in diabetes, we evaluated the activation of various types of PKC isoforms in normal and diabetic VSMCs. As shown in Figure 2B, PDGF-BB and diabetes did not cause any significant change in the cytosolic or the membranous fraction of PKC-α and PKC-βII. In contrast, without any stimulation, the PKC-βII and PKC-δ expressions in the membranous fraction were significantly increased to 204% and 189%, respectively, in diabetic cells compared with those in normal cells. Furthermore, by PDGF-BB, although the membranous level of PKC-βII was not changed in normal or diabetic cells, that of PKC-δ was significantly elevated in normal and diabetic cells.

Figure 3. Effects of GF109203X (A) and rottlerin (B) on PDGF-BB–induced p38 phosphorylation in normal and/or diabetic VSMCs. p-p38 indicates the expression of phosphorylated p38. The quantification of the level of phosphorylated p38 from 3 separate experiments is shown in the respective lower panel. A, *P<0.05 versus PDGF-BB(−) without GF109203X; †P<0.05 versus PDGF-BB(−) without GF109203X. B, *P<0.05 versus PDGF-BB(−) without rottlerin in normal cells; †P<0.05 versus PDGF(+) without rottlerin in normal cells; ¶P<0.05 versus PDGF(−) without rottlerin in diabetic cells; ‡P<0.05 versus PDGF(+) without rottlerin in diabetic cells.

Figure 3. Effects of GF109203X (A) and rottlerin (B) on PDGF-BB–induced p38 phosphorylation in normal and/or diabetic VSMCs. p-p38 indicates the expression of phosphorylated p38. The quantification of the level of phosphorylated p38 from 3 separate experiments is shown in the respective lower panel. A, *P<0.05 versus PDGF-BB(−) without GF109203X; †P<0.05 versus PDGF-BB(−) without GF109203X. B, *P<0.05 versus PDGF-BB(−) without rottlerin in normal cells; †P<0.05 versus PDGF(+) without rottlerin in normal cells; ¶P<0.05 versus PDGF(−) without rottlerin in diabetic cells; ‡P<0.05 versus PDGF(+) without rottlerin in diabetic cells.

Effect of PKC on PDGF-BB–Induced p38 Phosphorylation

As shown in Figure 3A, without any stimulation, the level of phosphorylated p38 was dose-dependently suppressed by GF109203X, a general PKC-speciﬁc inhibitor. Although the level of phosphorylated p38 was remarkably increased to ~3-fold by PDGF-BB, the elevation was also dose-dependently inhibited by GF109203X.

To clarify the role of PKC isoforms in PDGF-BB–induced p38 phosphorylation, we examined the effects of CGP-53353, a PKC-II/II–specific inhibitor, and rottlerin, a PKC-δ–specific inhibitor, on PDGF-BB–induced p38 phosphorylation in normal and diabetic VSMCs. CGP-53353 could not influence p38 phosphorylation in PDGF-BB or diabetes (data not shown). In contrast, as shown in Figure 3B, without any stimulation, 10 μmol/L of rottlerin could reduce the level of phosphorylated p38 in normal VSMCs. In addition, the
Because PDGF-BB-induced p38 phosphorylation was shown to be regulated by PKC-δ in Figure 3B, we examined the effects of the PKC-isoform and/or ROCK on the cell migration of normal and diabetic VSMCs. Without any stimulation, the value of the migration activity in diabetic cells significantly increased compared with that in normal cells; however, migration activity was prevented by rottlerin alone, but not by Y-27632 or CGP53533. Similarly, although the value of migration activity was more elevated by PDGF-BB in normal and diabetic VSMCs, the elevation was significantly suppressed by rottlerin alone (Figure 4B).

To demonstrate the crucial role of PKC-δ on PDGF-BB–induced cell migration in diabetic VSMCs, we examined the migration activity using antisense ODN specific for PKC-δ. After adding 0.2 to 10 μmol/L antisense PKC-δ ODNs, PKC-δ protein expression was maximally decreased at 1 μmol/L (data not shown). As shown in Figure 4C, the protein expression of PKC-δ was not decreased by 1 μmol/L of sense ODN, but it was decreased to 60% of the normal control by the antisense ODN. The levels of PKC-δ protein in diabetic cells were elevated to 132±17% of those of normal cells and inhibited to 66±13% of those of control cells when incubated with antisense PKC-δ ODN in diabetic cells. The inhibitory effect of antisense PKC-δ ODN did not affect the expression of PKC-α. Interestingly, as shown in Figure 4D, the PDGF-BB–induced migration activity in diabetic cells was significantly prevented by the antisense ODN for PKC-δ.

PDGF-BB–Induced p38 Phosphorylation Regulates Cell Growth and the Levels of COX-2 and Arachidonic Acid Release, but not Apoptosis

To characterize additional biological roles of PDGF-BB–induced p38 phosphorylation in normal and diabetic VSMCs, the association with cell growth and/or apoptosis was studied. As shown in Figure 5A, with or without PDGF-BB, the value of [3H]-thymidine incorporation in diabetic cells was more elevate than that in normal cells, and the increase was dose-dependently prevented by SB-203580. In contrast, by an in situ apoptosis assay using the TUNEL method, positive immunoreactivities in the nuclei were only seen in the anisomycin-treated cells. However, the nuclei from other groups showed no immunoreactivity with PDGF-BB or SB-203580 (data not shown).

In contrast, although the protein level of COX-1 was not affected by PDGF-BB in normal or diabetic cells (left panel of Figure 5B), the protein level of COX-2 in diabetic cells significantly increased compared with that in normal cells without any stimulation. Interestingly, the protein levels of COX-2 with PDGF-BB in normal and diabetic cells were higher than those without PDGF-BB. Similarly to the result of PDGF-BB–induced p38 phosphorylation, the elevated protein levels of COX-2 were inhibited by SB-203580 in cells (right panel of Figure 5B).

In addition, we examined whether PDGF-BB–induced p38 could affect the change of arachidonic acid release. Similarly, without PDGF-BB, the level of arachidonic acid release in diabetic cells significantly increased compared with that in normal cells, and PDGF-BB enhanced these releases in both
et al reported that the proliferative characteristics is observed in diabetic rat VSMCs. PDGF-BB–induced p38 phosphorylation remarkably increased in both normal and diabetic cells. PDGF-BB–induced elevations were also suppressed by SB-203580 (Figure 5C).

Discussion

This study clearly demonstrated that PDGF-BB phosphorylated p38 and the phosphorylation remarkably increased in diabetic rat VSMCs. PDGF-BB–induced p38 phosphorylation was mediated by PKC-δ and the subsequent MKK 3/6, independently of MEK 1/2, ROCK, or PI3K, in normal and diabetic cells. PDGF-BB–induced p38 phosphorylation regulated cell growth and migration, COX-2 expression, and arachidonic acid release, which were remarkably enhanced in diabetes. These results strongly suggested that p38 could be one of the most crucial targets to prevent the progression of atherosclerosis and its related chronic inflammatory process in diabetic vasculature.

In this study, an enhanced growth property in diabetic VSMCs was observed until the fifth passage compared with normal cells, suggesting that diabetes itself could alter the various signaling transduction pathways. Kanzaki et al recently showed that a latent transforming growth factor (TGF)-β1–binding protein stimulates the migration of STZ-induced diabetic VSMCs through increased expression of integrin-β3 more than that of normal cells in vitro. Pandolfi et al reported that the proliferative characteristics is observed in diabetic VSMCs between 4 and 8 passages and the properties are associated with a redox imbalance responsible quenching and/or trapping of nitric oxide by glucose and its related metabolites. These findings may support the possibility that diabetes can bring about some phenotypic change in VSMCs. As one consequence of the phenotypic change in diabetic VSMCs, as shown in another report, the level of the PDGF β-receptor was remarkably enhanced compared with that in normal rat cells. Because PDGF-BB modulates the intracellular signaling pathways through the PDGF β-receptor, changes in such VSMC properties could play an important role in accelerating the early events of atherosclerosis in diabetes.

Although we and other investigators have shown that PDGF-BB can phosphorylate p38 in normal VSMCs, PDGF-BB–induced p38 phosphorylation in diabetic cells has not been evaluated. Interestingly, in this study, we could show that PDGF-BB–induced p38 phosphorylation in diabetic cells was more elevated than that in normal cells. Possibly, the enhancement of PDGF-BB–induced p38 phosphorylation in diabetic cells may result from the phenotypic change caused by diabetes, including the overexpression of the PDGF β-receptor. In addition, even without PDGF-BB, the phosphorylated p38 level in diabetic cells already increased more than that in normal cells. Although the precise mechanism is obscure, it is very likely that some phenotypic changes resulting from diabetes might be related to p38 phosphorylation, because diabetes causes a variety of pathologic changes in vasculature.

In the upstream level of p38, PDGF-BB could specifically phosphorylate p38 via the MKK 3/6-dependent pathway, independently of the MEK-ERK cascade, or the MKK 4 or its downstream, JNK, even in diabetic VSMCs. In addition, notably, PDGF-BB could enhance the membranous fraction level of PKC-δ alone in normal and diabetic cells. As for PDGF-BB–induced PKC activation, in agreement with our finding, Saito et al have shown that PKC-δ could be activated through a PDGF β-receptor. In contrast, Carlin et al reported that PKC-ζ, but not PKC-δ, was associated with PDGF-induced cell proliferation. Although the precise mechanism is obscure, the discrepancy may be based on difference in experimental condition or the essential properties of the stimulants, because PDGF-BB–induced PKC-ζ activation was not observed in this study (data not shown). GF109203X dose-dependently inhibited the level of phosphorylated p38 with or without PDGF-BB in normal VSMCs. In addition, only rottlerin, but not other inhibitors, dose-dependently inhibited the phosphorylated p38 level, with or without PDGF-BB, in diabetic cells. Therefore, the activation of PKC-δ, but not that of PKC-ζ, ROCK, or PI3K, could very likely play an important role in PDGF-BB–induced p38 phosphorylation in diabetic VSMCs.

As important biological consequences, as in previous reports, we clearly demonstrated that PDGF-BB dose-dependently activated cell migration via the p38 pathway, but not the MEK-ERK pathway, in normal VSMCs. Interestingly, in diabetic VSMCs, PDGF-BB–induced cell migration was more elevated; the elevation was significantly inhibited by rottlerin and antisense ODN for PKC-δ, but not by Y-27632...
or CGP53353. These findings demonstrated that PDGF-BB–induced cell migration was regulated by p38 coupling with PKC-δ activation, independently of ROCK or other PKC isoforms. In contrast, PDGF-BB–induced VSMC migration was inhibited at only 2 ng/mL, but not at higher concentrations, by wortmannin, suggesting that PI3K may not be mainly associated with regulating cell migration by PDGF-BB–induced p38 phosphorylation.

This study also demonstrated that PDGF-BB–induced p38 phosphorylation may be related to cell growth. However, in the TUNEL method, the nuclei of PDGF-BB–stimulated VSMCs did not show apoptotic change, in contrast to those stimulated by anisomycin. Therefore, PDGF-BB–induced p38 phosphorylation in diabetic VSMCs could contribute to an alteration in cell growth, probably through β receptors, that is essentially different from activation by any environmental stress factor or proinflammatory cytokine. In contrast, another report showed that p38 did not affect PDGF-BB–stimulated DNA synthesis in porcine aortic endothelial cells overexpressing tyrosine residue-mutated PDGF α-receptors or PDGF β-receptors. It is plausible that the discrepancy results from using different cell types or culture conditions, ie, endothelial cells and 20 ng/mL of PDGF-BB, an extremely high concentration that cannot be inhibited by any inhibitor, including SB-203580.

Interestingly, PDGF-BB–induced p38 phosphorylation could regulate the levels of COX-2 and arachidonic acid release, which were synergically increased by PDGF-BB and the diabetic condition. Hayama et al have shown that PDGF-BB and H2O2–related p38 activation is involved in cytosolic phospholipase A2 activation and arachidonic acid release in mesangial cells. Lin et al reported that tumor necrosis factor-α–induced COX-2 expression is partially mediated through p38, leading to prostaglandin production in tracheal smooth muscle cells. These findings may support the possibility that PDGF-BB–induced p38 phosphorylation may influence the chronic inflammatory process in the progression of atherosclerosis, especially in diabetic patients.

In conclusion, our study established that PDGF-BB phosphorylated p38 via PKC-δ and the subsequent M KK 3/6 cascade and that the phosphorylation could regulate cell growth and the progression of the chronic inflammatory process in the diabetic vascular wall. These results provide a new insight into the potential cellular and molecular mechanisms whereby the suppression of p38 activation may have clinical benefits and contribute to the prevention of atherosclerosis, especially in diabetic patients.

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References


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Figure Legends

Figure I.  (A)VSMC outgrowth rate from aortic explant of normal and diabetic rat. *p<0.05 vs. normal(n=7, respectively).  (B)Cell growth curves in the 5th passage of normal and diabetic VSMCs. *p<0.05 vs. Normal(n=4, respectively).  (C)Effects of PDGF-BB and SB-203580 on p38 phosphorylation in normal and diabetic VSMCs.  p-p38 indicates the expression of phosphorylated p38. The quantification of the level of phosphorylated p38 from three separate experiments is shown in the lower panel. *p<0.05 vs. PDGF-BB(-) without SB-203580 in normal cells; #p<0.05 vs. PDGF(+) without SB-203580 in normal cells; †p<0.05 vs. PDGF(+) without SB-203580 in diabetic cells.  (D)Effects of PDGF-BB on MKK 3/6 phosphorylation.  p-MKK 3/6 indicates the expression of phosphorylated MKK 3/6. The quantification of the phosphorylated levels from four separate experiments is shown in the lower panels. *p<0.05 vs. PDGF-BB(-) in normal cells; #p<0.05 vs. PDGF(+) in normal cells; ¶p<0.05 vs. PDGF(-) in diabetic cells.  (E)Effects of PDGF-BB and PD-98059 on p38 phosphorylation in normal and diabetic VSMCs.  p-p38 indicates the expression of phosphorylated p38. The quantification of
phosphorylated p38 from three separate experiments is shown in the lower panel.

*p<0.05 vs. PDGF-BB(-) in normal cells; #p<0.05 vs. PDGF(+) in normal cells; ¶p<0.05 vs. PDGF(-) in diabetic cells.

**Figure II.** (A)*In situ* PKC activity in normal and diabetic VSMCs. The assay was performed in duplicate (n=6, respectively). *p<0.01 vs. PDGF-BB(-) in normal cells; #p<0.01 vs. PDGF(-) in diabetic cells. (B)Effect of PDGF-BB on the activation of PKC isoforms in normal and diabetic VSMCs. The quantification of the level of each PKC isoform from four separate experiments is shown in the lower panel. *p<0.05 vs. PDGF-BB(-) in normal cells; #p<0.05 vs. PDGF(-) in diabetic cells.

**Figure III.** Effects of GF109203X(A), and rottlerin(B) on PDGF-BB-induced p38 phosphorylation in normal and/or diabetic VSMCs. p-p38 indicates the expression of phosphorylated p38. The quantification of the level of phosphorylated p38 from three separate experiments is shown in the respective lower panel. (A), *p<0.05 vs. PDGF-BB(-) without GF109203X; †p<0.05 vs. PDGF(+) without GF109203X. (B),
\*p<0.05 vs. PDGF-BB(-) without rottlerin in normal cells; \#p<0.05 vs. PDGF(+) without rottlerin in normal cells; ¶p<0.05 vs. PDGF(-) without rottlerin in diabetic cells; †p<0.05 vs. PDGF(+) without rottlerin in diabetic cells.

**Figure IV.** (A) Effects of PDGF-BB and various types of inhibitors on the migration activity in normal VSMCs. *p<0.01 vs. PDGF-BB(-) without inhibitor; #p<0.05, ##p<0.01 vs. the same concentration of PDGF-BB(+) without inhibitor(n=6). (B) Effects of PDGF-BB and various types of inhibitors on the migration activity in normal and diabetic VSMCs. *p<0.05 vs. PDGF-BB(-) without an inhibitor in normal cells; #p<0.05 vs. PDGF(-) without an inhibitor in diabetic cells; ¶ p<0.05 vs. PDGF(+) without an inhibitor in diabetic cells(n=6). (C) Effect of antisense oligodeoxynucleotides(ODN) for mRNA specific for human PKC-δ isoform on the expression of PKC-δ and -α in normal and diabetic VSMCs. The quantification of the protein level of PKC-δ from four separate experiments is shown in the lower panel. *p<0.05 vs. lipofectamine alone in normal cells; #p<0.05 vs. lipofectamine alone in diabetic cells. (D) Effects of PDGF-BB and antisense ODN for mRNA specific for the human PKC-δ isoform on the migration activity in normal
and diabetic VSMCs. †p<0.05 vs. PDGF(+) with lipofectamine alone in diabetic cells (n=6).

Figure V. Effects of PDGF-BB and SB-203580 on DNA synthesis (A), the expression of COX-1 and COX-2 (B), and arachidonic acid release (C) in normal and diabetic VSMCs. (A) and (B) were performed in duplicate (n=4 in each case), and (C) was obtained from three separate experiments. (A), *p<0.05, **p<0.01 vs. PDGF-BB(-) without SB-203580 in normal cells; #p<0.05, ##p<0.01 vs. each condition without SB-203580. (B) and (C), *p<0.05, **p<0.01 vs. PDGF-BB(-) without SB-203580 in normal cells; #p<0.05 vs. PDGF(+) without SB-203580 in normal cells; †p<0.05, ††p<0.01 vs. PDGF(-) without SB-203580 in diabetic cells; §p<0.05 vs. PDGF-BB(+) without SB-203580 in normal cells; ¶p<0.05 vs. PDGF(+) without SB-203580 in diabetic cells.
Figure I
Figure II

(A) In situ PKC Activity

PKC-\(\alpha\)
PKC-\(\beta\)
PKC-\(\beta\)I
PKC-\(\beta\)II

PDGF-BB (5 ng/ml)

In situ PKC Activity (pmol/min/mg protein)

N : Normal; DM : Diabetic

(B) Membrane and Cytosol PKC Isoforms

PKC-\(\alpha\)
PKC-\(\beta\)
PKC-\(\beta\)I
PKC-\(\beta\)II
PKC-\(\delta\)

Optical Density Units of Membranous Fraction [% of N (-)]

N : Normal; DM : Diabetic
Figure III
Figure IV
Figure V

(A) 

[Graph showing the effect of SB-203580 on [3H] Thymidine Incorporation. The x-axis represents SB-203580 (μM) and the y-axis represents [3H] Thymidine Incorporation (% of Normal). Data points are shown for Normal and Diabetic groups with and without SB-203580.]

(B) 

[Graph showing the effect of PDGF-BB and SB-203580 on COX-1 and COX-2 expression. The x-axis represents PDGF-BB (1 ng/ml) and SB-203580 (1 μM), and the y-axis represents Optical Density Units (% of control). Data points are shown for Normal and Diabetic groups with and without PDGF-BB and SB-203580.]

(C) 

[Graph showing the effect of PDGF-BB and SB-203580 on Arachidonic Acid Release. The x-axis represents PDGF-BB (5 ng/ml) and SB-203580 (1 μM), and the y-axis represents Arachidonic Acid Release (% of Control). Data points are shown for Normal and Diabetic groups with and without PDGF-BB and SB-203580.]