

Ca²⁺-channel blockers inhibit the action of recombinant platelet-derived growth factor in vascular smooth muscle cells

(phosphatidylinositol turnover)

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ABSTRACT Human platelet-derived growth factor (PDGF) is mainly composed of two polypeptide chains (PDGF-AB). All three possible dimeric forms of PDGF—i.e., PDGF-AA, PDGF-BB and PDGF-AB—exist in nature. We have used two recombinant PDGF homodimers to determine the roles of each isoform in the activation of phosphatidylinositol turnover in vascular smooth muscle cells (VSMC) isolated from rat thoracic aorta, their mitogenic effect on VSMC, and their vasoconstrictor effect on intact strips of aortic vascular tissue. Three Ca²⁺-channel blockers, nifedipine, verapamil, and diltiazem, were used as antagonists for investigating the PDGF-dependent changes mediated by the homodimers. PDGF-BB had a greater efficacy than PDGF-AA on inositol 1,4,5-trisphosphate release, on the formation of diacylglycerol, and on Ca²⁺ mobilization, which was also associated with vasoconstrictor activity and effective mitogenicity. PDGF-AA, on the other hand, was more potent than PDGF-BB in stimulating protein kinase C. In all instances, the activation of the phosphatidylinositol turnover by the two homodimers was inhibited by the Ca²⁺-channel blockers.

Platelet-derived growth factor (PDGF) is a major mitogen in serum for connective tissue-derived cells as well as for glial cells (1, 2). Structurally, it is a dimer of two different homologous polypeptide chains, commonly denoted PDGF-AB. Its activity is achieved by its binding to specific cell surface receptors; this leads to certain physiological changes, including receptor autophosphorylation and phosphorylation of tyrosine residues of certain cytoplasmic substrates (3, 4). PDGF stimulates the turnover of phosphatidylinositol (PtdIns), which includes the formation of inositol 1,4,5-trisphosphate (InsP₃) and diacylglycerol (DAG) associated with a rise in the cytoplasmic concentration of free calcium, [Ca²⁺]_i, and activation of protein kinase C (PKC). Recently, it has been shown, using rat aortic tissue, that PDGF-AB is a potent vasoconstrictor (5).

Ca²⁺-channel blockers lower blood pressure (6) and increase the hydrolysis of cholesterol esters in vascular smooth muscle cells (VSMC) (7). In addition, they inhibit the mitogenic effects of Ca²⁺ agonists in arterial smooth muscle cells (8). Berk *et al.* (5) reported that platelet PDGF-AB, in part, mobilizes Ca²⁺ from intracellular sources and causes an influx of Ca²⁺ in VSMC. Since Ca²⁺-channel blockers inhibit Ca²⁺ influx, it is conceivable that they affect the action of PDGF. In view of the fact that PDGF-AB mobilizes Ca²⁺, thereby increasing [Ca²⁺]_i, which participates in a contractile response in vascular tissue, do each of the polypeptide chains, A and B, of the heterodimer, regulate specific cellular activities? With this in mind, we have constructed, with

recombinant cloning techniques in *Escherichia coli*, PDGF homodimers: PDGF-AA and PDGF-BB.

We report here (i) that recombinant PDGF-AA and PDGF-BB molecules have distinct abilities to stimulate the PtdIns turnover in VSMC and that the mitogenic and vasoconstrictor responses to the PDGF-BB are far greater than those of PDGF-AA; (ii) concomitantly, these effects are accompanied by an increased formation of InsP₃ and DAG, associated with a rise in [Ca²⁺]_i; (iii) in contrast, PDGF-AA has a higher efficacy for activating PKC; and (iv) these events are inhibited by Ca²⁺-channel-blocking agents.

METHODS

Cell Isolation and Cell Cultivation. VSMC were isolated from rat aorta and cultured for several passages in the manner described by Ross (9). Segments of the thoracic aorta from normotensive rats (Wistar Kyoto strain) were obtained from female rats that were 6–8 weeks of age. All procedures were carried out under sterile conditions. The thoracic aorta was removed and placed in a Petri dish (Falcon) that contained ice-cold Dulbecco's modified Eagle's medium (DMEM) (GIBCO). The intima and the outer and inner tissue layers were removed carefully; the VSMC tissue layer was cut into approximately 1-mm squares, which were transferred into a 25-cm² tissue culture flask (Falcon) and barely covered with DMEM that was supplemented with 10% fetal calf serum (GIBCO), nonessential amino acids, and penicillin and streptomycin (each 100 μg/ml). The blocks of tissue were cultured at 37°C in a humidified atmosphere of 95% air and 5% CO₂. The cells became confluent after approximately 3–4 weeks. From these cells, subcultures were prepared as described by Ross (9) and Ross and Glomset (10). VSMC were used between the fourth and eighth passage. The samples were examined with electron and fluorescence microscopes. To ensure the authenticity of the subcultured cells, immunofluorescence staining of appropriate marker proteins was routinely performed.

Preparation of Recombinant Homodimers (PDGF-AA and PDGF-BB). A detailed description is given in ref. 11. In brief, sequences coding for the mature PDGF-A and -B were fused in-frame into a truncated *cro'-lacZ* gene in the vector pEx-1. For PDGF-A the arginine residue at position -1 was mutated into a methionine by site-directed mutagenesis. From the expressed fusion proteins the PDGF-containing sequences were excised by CNBr cleavage, which led in the case of PDGF-B to a protein devoid of the NH₂-terminal 12 amino

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Abbreviations: PDGF, platelet-derived growth factor; PtdIns, phosphatidylinositol; InsP₃, inositol 1,4,5-trisphosphate; DAG, diacylglycerol; VSMC, vascular smooth muscle cells; PMA, phorbol 12-myristate 13-acetate; PKC, protein kinase C.

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acid residues. After protection of thiol residues by S-sulfonation, monomeric proteins were isolated by gel permeation chromatography in 4 M guanidine-hydrochloride followed by reverse-phase high-performance liquid chromatography. Dimerization was induced by treatment with a mixture of reduced and oxidized glutathione in the presence of urea. Final purification was achieved by ion-exchange chromatography. The renatured recombinant PDGF-AA showed a mitogenic potency similar to that obtained from yeast cells ($ED_{50} = 12 \text{ ng/ml}$) (3). The mitogenic activity of recombinant PDGF-BB on mouse AKR-2B fibroblasts exhibited an ED_{50} of 1.5 ng/ml, which was slightly lower than that for PDGF prepared from human platelets ($ED_{50} = 3.3 \text{ ng/ml}$) (11).

Measurement of Mitogenicity. The mitogenic effect was measured by the incorporation of [^3H]thymidine into VSMC, according to the protocol of Chesterman *et al.* (12).

Studies of Recombinant PDGF on the Contractile Response of Isolated Rat Aorta. Strips of rat aorta were prepared and were allowed to equilibrate for 2 hr in an appropriate tissue bath, as described by Berk *et al.* (13). The strips of aorta had intact endothelium (this was assessed by vasodilation when challenged with acetylcholine). The strips were placed at an optimal length for the development of active force in response to norepinephrine. Contractile responses to PDGF-AA or PDGF-BB were determined after sequential addition of increasing amounts of the agonist into the tissue bath. Concentrations are expressed as cumulative molar concentrations.

Effect of Recombinant PDGF-BB on $[\text{Ca}^{2+}]_i$. Confluent VSMC were harvested and were washed twice with Hepes buffer solution (3 mM sodium Hepes/16 mM glucose/133 mM NaCl/1 mM MgSO_4 /0.5 mM CaCl_2) containing 1% bovine serum albumin. The cells were then cultured onto glass microscope slides (26 × 76 mm) under normal tissue culture conditions. When the cells became confluent (20,000–40,000 cells per cm^2), they were washed in Hepes buffer that contained 1% bovine serum albumin. They were incubated with fura-2 acetoxyethyl ester (2 μM) for 30 min in a 37°C shaking water bath. Just prior to the measurements, the monolayer of cells was rinsed with NaHepes buffer without bovine serum albumin but with 1 mM CaCl_2 . The glass slide was positioned at the appropriate angle in the cuvette by a custom-made adapter [previously described (14)]. After the addition of the agonists, cells containing fura-2 were analyzed in a SLM-Aminco SPF 500 spectrofluorophotometer. Fluorescence was measured at 37°C with a beam-splitter, two excitation monochromators, and a dual mirror chopping mechanism, which allowed the rapid alternating excitation of fura-2 at two wavelengths. Excitation wavelengths were set at 346 and 373 nm and the emission wavelength chosen was 510 nm. Data were collected with an IBM personal computer that was coupled to the spectrofluorometer. The 340/380-nm ratio of the emitted fluorescence signal permits calculation of the cytosolic free calcium. Calibration of the fluorescence signal was performed as described by Grynkyewicz *et al.* (15).

Determinations of InsP_3 and DAG. InsP_3 and DAG were determined as described previously (16, 17).

PKC Assay. The subcultured VSMC were transferred to a 30-mm plastic Petri dish (Falcon); they were incubated for 3 days in DMEM supplemented with 10% fetal calf serum. The cells (3×10^6 per dish) were then rinsed extensively with serum-free DMEM and then stimulated with PDGF-AA or PDGF-BB. After stimulation, the cells were incubated at 37°C in a humidified atmosphere of 5% CO_2 and 95% air. The reactions were stopped at the indicated times by placing the Petri dish on ice, decanting the supernatant fluid, and adding 3 ml of ice-cold sample buffer (20 mM Tris-HCl, pH 7.5/0.33 M sucrose/2 mM EDTA/0.5 mM EGTA/2 mM phenylmeth-

ylsulfonyle fluoride). The VSMC were then collected with a rubber policeman and were disrupted by sonication (two 30-sec treatments). The solution, which contained cytosol and membrane components, was centrifuged at $100,000 \times g$ for 60 min and was processed further, as described by Farrar *et al.* (18). Aliquots (50 μl) were assayed for PKC activity with 1 mM CaCl_2 , 10 mM magnesium acetate, 100 μM [$\gamma\text{-}^{32}\text{P}$]ATP (60 cpm/pmol), and 50 μg of histone H1, with or without 5 μg of phosphatidylserine and 1 μg of dioleoylglycerol. Data are expressed as PKC specific activity (pmol/min per mg of protein) attributed to activity stimulated by phospholipid.

RESULTS

Effect of Ca^{2+} -Channel Blockers on Recombinant PDGF-Dependent Mitogenic and Vasoconstrictor Responses. The strength of the vasoconstrictor activity of PDGF-BB ($ED_{50} = 6.8 \times 10^{-11} \text{ M}$) on rat aortic strips was about twice that of PDGF-AA (Fig. 1). The response to both agonists increased as a function of concentration. The endothelium of the aortic vascular strips was intact, since its integrity was confirmed by inducing relaxation of the smooth muscle strips with acetylcholine. Moreover, after the removal of the endothelium, there was no change in the maximum response in the presence of the agonists. This excluded an interaction between the endothelium and the agonists responsible for the vasoconstrictor response. In contrast, when aortic strips with denuded endothelium were tested in the presence of norepinephrine plus PDGF, their vasoconstrictor response was increased. In addition, depletion of extracellular Ca^{2+} abolished the contractile response induced by the two agonists. When the aortic strips were incubated with any one of the three Ca^{2+} antagonists (verapamil, nifedipine, or diltiazem) at a constant concentration of 5 μM , the vasoconstrictor re-

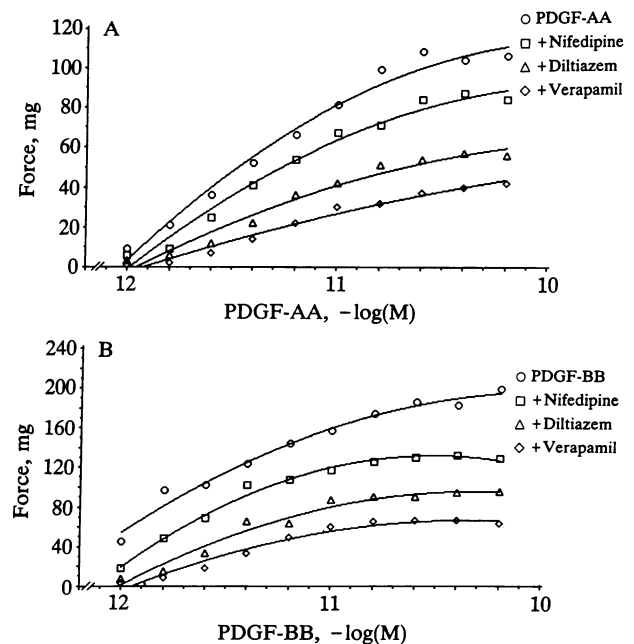


Fig. 1. Effect of Ca^{2+} -channel blockers on contractile activity of isolated rat aorta induced by PDGF-AA (A) or PDGF-BB (B). Strips of rat aorta were prepared and allowed to equilibrate for 2 hr in an appropriate tissue bath, as described by Berk *et al.* (13). The strips were placed at an optimal length for the development of active force in response to norepinephrine. Contractile response to increasing concentrations of either PDGF-AA or PDGF-BB was determined after incubation of the strips with one of the Ca^{2+} -channel blockers (5 μM) for 30 min (1 mg of force = 9.8 μN). The data are representative of six independent experiments.

sponse elicited by the addition of PDGF-BB or PDGF-AA was greatly diminished. Verapamil was the most potent agent, causing a more than 60% inhibition.

As described earlier, the two PDGF homodimers stimulated the incorporation of [³H]thymidine into quiescent smooth muscle cells (19). In this assay, too, PDGF-BB was more potent ($ED_{50} = 5$ ng/ml) than PDGF-AA ($ED_{50} = 15$ ng/ml) (Fig. 2). Likewise, the maximum incorporation stimulated by PDGF-BB was approximately 50% higher than that for PDGF-AA. Notably, as described by Berk *et al.* (5), the concentrations of both agents required for vasoconstriction were significantly lower than those required for growth promotion. Ca^{2+} antagonists effectively inhibited the stimulation of growth induced by PDGF-AA and -BB. Verapamil inhibited the PDGF-BB-dependent effect at $IC_{50} = 3.3 \times 10^{-7}$ M, diltiazem at $IC_{50} = 4.7 \times 10^{-7}$ M; nifedipine was the least effective at $IC_{50} = 5.4 \times 10^{-6}$ M.

Effect of Ca^{2+} -Channel Blockers on Recombinant PDGF-Dependent Formation of $InsP_3$ and DAG and Ca^{2+} Mobilization. The K_d for receptor binding obtained by a competition experiment using ¹²⁵I-labeled PDGF, prepared from human platelets, and various concentrations of the recombinant proteins was estimated to be 9 ± 1.3 ng/ml for PDGF-AA and 13.3 ± 2.2 ng/ml for PDGF-BB (data not shown).

In VSMC labeled with *myo*-[³H]inositol, the addition of PDGF-BB (100 ng/ml) resulted in a release of labeled inositol phosphate. The *myo*-inositol phosphate accumulation reached its maximum stimulation after approximately 5 min of incubation (Fig. 3A). Associated with the maximum formation of $InsP_3$ there was increased production of DAG (Fig. 3B). In contrast to the activity of the PDGF-BB homodimer, there was no significant response in the forma-

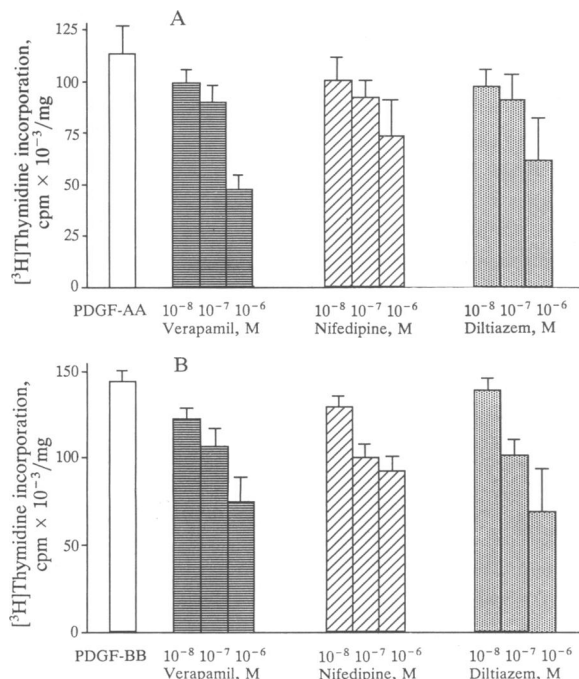


FIG. 2. Effect of Ca^{2+} -channel blockers on the mitogenic response of VSMC to either PDGF-AA (A) or PDGF-BB (B). The mitogenic activities for the recombinant proteins were 15 ng/ml (ED_{50} for PDGF-AA) and 5 ng/ml (ED_{50} for PDGF-BB). The mitogenic response of VSMC by either PDGF-AA or PDGF-BB (50 ng/ml), which in both cases causes maximum [³H]thymidine incorporation in the presence and absence of various concentrations of Ca^{2+} , was measured by using the protocol of Chesterman *et al.* (12). Basal values for nonstimulated controls were 1600 ± 480 cpm/mg of protein. The data are the mean \pm SD of five independent experiments.

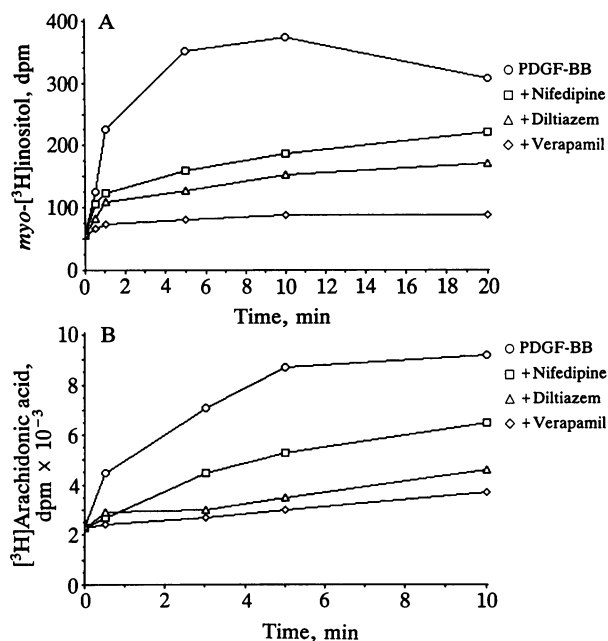


FIG. 3. (A) Time-dependency in the release of *myo*-[³H]inositol-labeled $InsP_3$ resulting from PDGF-BB stimulation of VSMC, in the presence and absence of Ca^{2+} -channel blockers. For determination of $InsP_3$, radiolabeled VSMC were exposed to PDGF-BB (100 ng/ml) for the time intervals indicated, after treatment of the cells with Ca^{2+} -channel blockers (5 μ M). In unstimulated VSMC the recovery of $InsP_3$ was 53 ± 29 dpm per 10^6 cells. (B) Effect of Ca^{2+} -channel blockers on PDGF-BB-induced formation of DAG by VSMC. VSMC labeled with [³H]arachidonic acid and incubated with a Ca^{2+} -channel blocker (5 μ M) for 1 hr were stimulated with PDGF-BB (100 ng/ml). Incubations were terminated at the times indicated, lipids were extracted, and [³H]DAG was quantified after thin-layer chromatography. Data are derived from six independent experiments.

tion of $InsP_3$ and DAG when PDGF-AA was used as the agonist at similar concentrations.

VSMC labeled with fura-2 and exposed PDGF-BB showed a rise in $[Ca^{2+}]_i$, which peaked at approximately 1 min and declined to base-line value within 5 min. In a calcium-free medium, at a similar concentration, PDGF-BB still caused an increase in $[Ca^{2+}]_i$, which suggests that it stimulates the release of Ca^{2+} from an intracellular source and causes an increased Ca^{2+} influx. PDGF-AA, at similar concentrations, was not effective (data not shown). The effect of Ca^{2+} -channel blockers at various concentrations on the recombinant PDGF homodimers (at a fixed concentration) with respect to the formation of $InsP_3$ and DAG and on the elevation of $[Ca^{2+}]_i$ demonstrated a rank order in their inhibitory potencies. Thus, as was observed in the vasoconstrictor assay, verapamil had a higher inhibitory potency than either nifedipine (Fig. 4) or diltiazem (data not given). This inhibition of Ca^{2+} mobilization by the Ca^{2+} -channel blockers could also be demonstrated when Ca^{2+} was depleted in the incubation medium (data not shown).

Effect of Ca^{2+} -Channel Blockers on Recombinant PDGF-Dependent Translocation of PKC. An increase in membrane-associated PKC activity of over 5-fold was observed within 10 min after PDGF-AA stimulation; this was followed by a rapid decline in the plasma membrane-associated activity, with a return to the base-line membrane specific activity by 16 min. Concomitant with the rapid increase of the membrane-associated activity, there was a decline in PKC activity in the corresponding cytosol fraction. Although a decline in the cytosolic activity could be detected as early as 1 min after stimulation, the lowest PKC activity was observed at approximately 10 min, corresponding to peak plasma mem-

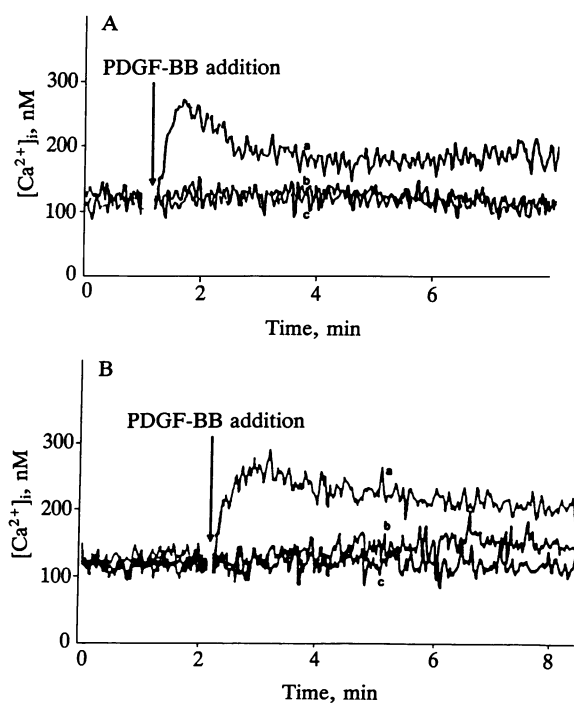


FIG. 4. (A) Effect of Ca^{2+} -channel blockers on PDGF-BB-induced elevation of $[\text{Ca}^{2+}]_i$ in VSMC. PDGF-BB (100 ng/ml) was added to the VSMC at zero time in the absence of Ca^{2+} -channel blockers (trace a) or in the presence of verapamil (10^{-7} M in trace b or 5×10^{-6} M in trace c). (B) Effect of PDGF-BB (100 ng/ml) alone (trace a) or PDGF-BB plus nifedipine (10^{-7} M in trace b or 5×10^{-7} M in trace c). Fura-2 traces at the monolayers of VSMC were recorded in the presence of Ca^{2+} in the incubation medium to which the calcium antagonists were added 1 hr before addition of PDGF. Data are representative of nine independent experiments.

brane-associated activity. Cytosolic PKC activity progressively increased, thereafter, in the continuous presence of the agonist. Phorbol 12-myristate 13-acetate (PMA), which was used as a control at 60 ng/ml, stimulated a rapid association of PKC with particulate membrane (within 2 min). The PMA-stimulated membrane association of PKC was approximately 1.5-fold greater than that induced by PDGF-AA, and dissociation from the VSMC membranes was more protracted. Cytosolic PKC activity rapidly declined within 2 min after PMA stimulation and was depressed to base-line values throughout the experiment (data not shown). The efficacy of recombinant PDGF-AA was far superior to that of the other homodimer in translocating PKC into membrane particulate fraction. (The ED_{50} of PDGF-AA was 110 ± 27.4 ng/ml, $n = 6$; and that of PDGF-BB was 415 ± 67.3 ng/ml, $n = 5$). The influence of Ca^{2+} -channel blockers on the PDGF-induced translocation was tested by incubation of the VSMC with the drugs before the assay was performed. The inhibitory effect of Ca^{2+} -channel blockers on the translocation of PKC induced by either PDGF-AA or PDGF-BB was dependent on the concentration of the drugs (Table 1). Verapamil ($\text{IC}_{50} = 6.2 \times 10^{-7}$ M) was more potent than nifedipine ($\text{IC}_{50} = 9.3 \times 10^{-7}$ M). In contrast, diltiazem, even when tested at 10^{-5} M, was not effective.

DISCUSSION

Our results demonstrate that important functional changes in VSMC—i.e., vasoconstriction and growth stimulated by the mitogen PDGF—are inhibited by Ca^{2+} -channel-blocking agents. Since it is thought that these changes are mediated by changes in the concentrations of InsP_3 , DAG, and Ca^{2+} and in the activation of PKC, we investigated the effects of three

Table 1. Effect of Ca^{2+} -channel blockers on recombinant PDGF-induced association of PKC with plasma membrane

Agonist	PKC activity, pmol/min per mg of cell protein	
	Membrane fraction	Cytosol fraction
Control	1,210 \pm 340	5,400 \pm 710
PMA (60 ng/ml)	10,520 \pm 150	800 \pm 430
PDGF-AA (200 ng/ml)	5,710 \pm 820	1,130 \pm 500
+ Verapamil (10^{-8} M)	4,320 \pm 460	2,240 \pm 120
+ Verapamil (10^{-7} M)	3,280 \pm 570	3,120 \pm 410
+ Verapamil (10^{-6} M)	2,950 \pm 400	3,400 \pm 340
+ Nifedipine (10^{-8} M)	4,830 \pm 600	1,800 \pm 210
+ Nifedipine (10^{-7} M)	3,910 \pm 530	2,710 \pm 230
+ Nifedipine (10^{-6} M)	3,710 \pm 410	3,820 \pm 400
+ Diltiazem (10^{-8} M)	5,220 \pm 700	1,410 \pm 320
+ Diltiazem (10^{-7} M)	4,980 \pm 520	1,900 \pm 240
+ Diltiazem (10^{-6} M)	4,660 \pm 670	1,700 \pm 320
PDGF-BB (500 ng/ml)	4,410 \pm 730	2,260 \pm 410
+ Verapamil (10^{-8} M)	4,110 \pm 430	2,440 \pm 320
+ Verapamil (10^{-7} M)	3,690 \pm 570	2,930 \pm 220
+ Verapamil (10^{-6} M)	3,170 \pm 350	3,430 \pm 170
+ Nifedipine (10^{-8} M)	4,220 \pm 210	2,330 \pm 320
+ Nifedipine (10^{-7} M)	3,820 \pm 520	2,620 \pm 210
+ Nifedipine (10^{-6} M)	3,370 \pm 330	3,120 \pm 420
+ Diltiazem (10^{-8} M)	4,310 \pm 530	2,360 \pm 380
+ Diltiazem (10^{-7} M)	4,280 \pm 220	2,470 \pm 110
+ Diltiazem (10^{-6} M)	4,350 \pm 330	2,570 \pm 310

The VSMC were exposed to PDGF-AA, PDGF-BB (each at the concentration giving half-maximal effect), or PMA for 10 min and processed. After treatment of the cells with Ca^{2+} -channel blockers at the indicated concentrations for 1 hr and addition of the agonists for 10 min, cytosol and particulate membrane fractions were prepared, and PKC was isolated by column chromatography. Aliquots of the fraction containing the enzymatic activity were assayed. Results are expressed as PKC specific activity (the increment of enzymatic activity due to stimulation by phospholipid) and represent the mean \pm SD of six independent determinations performed in duplicate.

Ca^{2+} -channel-blocking agents on these events. We have used recombinant PDGF-AA and -BB homodimers, which have recently been shown to act via different pathways. Although the homodimers act quite differently on the investigated properties, all effects could be suppressed by Ca^{2+} -channel-blocking agents.

PDGF-BB seems to act via the established pathway of signal transduction—i.e., the stimulation of InsP_3 and DAG formation, $[\text{Ca}^{2+}]_i$ elevation, and to some extent PKC translocation.

VSMC are known to express both potential- and receptor-operated channels. While studies of the action of Ca^{2+} -channel blockers in VSMC have revealed high-affinity binding sites for dihydropyridines and phenylalkylamines (reviewed in ref. 20), their possible influence on receptor-operated channels is yet undefined. However, evidence for direct and rapid entry of extracellular Ca^{2+} into the agonist-regulated pool in VSMC has been reported by Casteels and Droogmans (21); they have also provided evidence that such a pathway may contribute to receptor-regulated Ca^{2+} entry. Though it is presently unclear how Ca^{2+} channels are linked to PDGF receptors, the observed decrease in $[\text{Ca}^{2+}]_i$, in the presence of the drugs, could be achieved by a modulation of PDGF-BB receptors. Similarly, Ca^{2+} -channel blockers were shown to inhibit the binding of platelet-activating factor to corresponding sites on platelets (22).

Similar to the observation of Berk *et al.* (5), who investigated the effect of native PDGF-AB, we also observed a PDGF-BB-dependent elevation of $[\text{Ca}^{2+}]_i$ in VSMC as a result of both an increased influx and an increased release of

Ca²⁺ from intracellular depots. The latter is thought to be dependent on the action of InsP₃, which acts as a second messenger that triggers the release of Ca²⁺ from an internal pool (23). Recently, Putney (24) and Irvine and Moor (25) reported that both a receptor-regulated Ca²⁺ entry and release of Ca²⁺ from internal stores are achieved by the action of InsP₃. Thus, InsP₃ promotes a release of Ca²⁺ from intracellular stores that depletes that pool and, in addition, InsP₃ serves as a signal that leads to the pool's refilling from the extracellular space. It is tempting, therefore, to conclude that the observed inhibition of a PDGF-BB-dependent Ca²⁺ mobilization in the VSMC may be mediated by the reduction in the amount of InsP₃, thereby inhibiting Ca²⁺ entry. Moreover, as was recently observed in cardiac tissue, phenylalkylamines bind to receptor sites in the sarcoplasmic reticulum (26). Analogously, when Ca²⁺ was depleted in the incubation medium, the PDGF-BB-dependent rise in [Ca²⁺]_i was found to be inhibited by the Ca²⁺-channel blockers. Thus, a direct effect of Ca²⁺-channel blockers on intracellular calcium depots, with resultant changes in the permeability of the organelles to Ca²⁺, has also to be taken into consideration.

Whetton *et al.* (27) have reported that interleukin 3 stimulates proliferation of a cell line via activation of PKC without increasing InsP₃. Analogously, our data show that PDGF-AA was a more potent activator of PKC, by far, without affecting InsP₃, but, surprisingly, this effect was inhibited by Ca²⁺-channel-blocking agents. An inhibitory action of the Ca²⁺-channel-blocking agents could be explained as an inhibition of signal transduction, which is responsible for the translocation of the enzyme from a cytosolic to a membranous pool. Thus, Ca²⁺-blocking agents would have to interfere by a novel mechanism, with the activation of PKC—e.g., by changing the hydrophilic or hydrophobic interactions in the membrane of VSMC during enzyme activation. An inhibition of PKC activity was reported for verapamil, however, by Mori *et al.* (28).

In conclusion, Ca²⁺-channel blockers are capable of inhibiting the action of recombinant PDGF-AA and PDGF-BB by interfering with their stimulatory action on PtdIns turnover. In view of the potential impact of PDGF in the pathogenesis of atherosclerosis, inflammatory diseases, and cancer (2), the regulatory role of Ca²⁺-channel-blocking agents and their specificity of action will have to be determined through additional studies.

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