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Intracellular calcium transients are necessary for platelet-derived growth factor but not extracellular matrix protein–induced vascular smooth muscle cell migration

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Purpose: **Vascular smooth muscle cell (SMC) migration is a critical component of the hyperplastic response that leads to recurrent stenosis after interventions to treat arterial occlusive disease. We investigated the relationship between intracellular calcium ($[Ca^{2+}]_i$) and migration of vascular SMCs in response to platelet-derived growth factor (PDGF) and extracellular matrix (ECM) proteins.**

Methods: **Human saphenous vein SMCs were used for all experiments. SMC migration in response to agonists was measured with a microchemotaxis assay. A standard fluorimetric assay was used to assess changes in $[Ca^{2+}]_i$ in response to the various combinations of growth factors and ECM proteins.**

Results: **The calcium ionophore A23187 produced a rapid rise in $[Ca^{2+}]_i$ and a corresponding 60% increase in SMC migration, whereas chelation of $[Ca^{2+}]_i$ with BAPTA (1,2-*bis* [aminophenoxy] ethane-*N,N,N′,N′*-tetraacetic acid) produced a fivefold decrease in PDGF-induced chemotaxis, suggesting that $[Ca^{2+}]_i$ is both sufficient and necessary for SMC migration. Stimulation of SMCs with PDGF produced an early peak followed by a late plateau in $[Ca^{2+}]_i$. To establish a relationship between temporal fluctuations in $[Ca^{2+}]_i$ and SMC migration, SMCs were pretreated with caffeine and ryanadine, which eliminated the initial peak but not the late plateau in $[Ca^{2+}]_i$, and had no effect on chemotaxis in response to PDGF. Incubation of SMCs with nickel chloride eliminated the late plateau, but had no effect on the initial peak in $[Ca^{2+}]_i$, and reduced PDGF-stimulated migration by fivefold. We then evaluated the role of calcium in SMC migration induced by ECM proteins such as laminin, fibronectin, and collagen types I and IV. All four matrix proteins stimulated SMC migration, but none produced an elevation in $[Ca^{2+}]_i$. Moreover, preincubation of SMCs with caffeine and ryanadine or nickel chloride had no effect on ECM protein-induced chemotaxis.**

Conclusion: **$[Ca^{2+}]_i$ transients are necessary for PDGF but not ECM protein-induced SMC chemotaxis. Moreover, the ability of PDGF to stimulate vascular SMC migration appears dependent on influx of extracellular calcium through membrane channels. (J Vasc Surg 2004;40:351-8.)**

Clinical Relevance: **Recurrent stenosis after angioplasty or surgical bypass remains a significant challenge in treating vascular occlusive disease. In addition to growth factors, extracellular matrix (ECM) proteins may be potent agonists of this process. In this study we show that the influx of extracellular calcium is an important mechanism for platelet-derived growth factor–induced smooth muscle cell migration but not ECM-induced migration. Of note, in clinical trials calcium channel blockers failed to inhibit recurrent stenosis. Our data provide mechanistic insight to help explain this negative outcome in that therapies designed to inhibit restenosis depend on the effects of both growth factors and ECM proteins.**

Vascular smooth muscle cell (SMC) proliferation and migration and the production of extracellular matrix (ECM) contribute to the pathophysiology of intimal hyperplasia.[1] Because few SMCs reside in the intimal layer of human vessels, SMC migration is an essential component of the intimal process that leads to recurrent stenosis.[2] A variety of growth factors and ECM proteins act as agonists of SMC migration.[3,4] Platelet-derived growth factor (PDGF) is one of the most potent SMC chemoattractants.[5] However, ECM proteins also act as stimulants for SMC migration, and their potency can be equivalent to or greater than that of PDGF.[6] Furthermore, ECM proteins may interact in a synergistic manner with PDGF to enhance SMC migration, which suggests overlapping yet distinct mechanisms for induction of migration.[7]

Cell movement or migration is the result of a cyclic process of cellular attachment and detachment.[8] Cells bind to ECM at specific sites termed focal adhesion complexes.[9] Central to the focal adhesion is the integrin receptor, which has a large extracellular domain, a membrane-spanning domain, and a short cytoplasmic domain. The extracellular

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domain attaches to matrix proteins specific for each integrin. On the cytoplasmic side, integrins interact with the actin cytoskeleton through a series of interconnecting proteins. Actin filaments are visible as stress fibers that form a framework or a structural skeleton that supports and shapes the cell. Movement of a cell is an active process for which there is a constant requirement for actin reorganization and focal adhesion complex assembly and disassembly. The net effect of this cyclic process is cellular locomotion.

There is substantial evidence in a number of cell types that calcium has an essential role in migration. Fluctuations in intracellular calcium ($[Ca^{2+}]_i$) known as calcium transients are focally and temporally associated with migrating eosinophils[10] and neutrophils.[11] Calcium transients have also been observed in human vascular SMCs migrating in response to serum[12] and after mechanical injury.[13] There are multiple mechanisms through which calcium may facilitate migration. Voltage-gated calcium channels mediate changes in $[Ca^{2+}]_i$ by enabling influx of extracellular ion and G protein–coupled release of calcium from the sarcoplasmic reticulum.[14] Free $[Ca^{2+}]_i$ binds calmodulin, which leads to activation of calmodulin-dependent protein kinase (CAMK). Pauley et al[15] have shown in vascular SMCs that inhibition of CAMK-II results in a 90% reduction in PDGF-stimulated migration. Thus CAMK-II may be the central pathway through which calcium influences SMC migration. Calcium also appears important for actin assembly, and acts as a cofactor for proteins associated with focal adhesions. Whether $[Ca^{2+}]_i$ is necessary for migration of human SMCs in response to PDGF and ECM proteins is not known. Moreover, the source of $[Ca^{2+}]_i$ and the timing of its release after stimulation with agonists of migration has not been explored.

MATERIAL AND METHODS

Reagents. Dulbecco modified Eagle medium (DMEM), fetal bovine serum, phosphate-buffered saline solution, *N*-2-hydroxyethylpiperazine-*N*-2-ethane sulfonic acid (HEPES), antibiotic-antimycotic, and trypsin–ethylenediamine tetraacetic acid (EDTA) were obtained from GIBCO BRL Life Technologies. Recombinant human PDGF-AB and PDGF-BB were obtained from Upstate Biotechnologies. Anti-SMC actin, bovine fibronectin (Fn), Engelberth-Holm Swarm mouse sarcoma laminin (Lm), calfskin collagen I (CNI), human placental collagen IV (CNIV), nickel chloride ($NiCl_2$), and caffeine were acquired from Sigma. Ionophore A23187 and ryanodine were purchased from Calbiochem. Fura-2-acetoxymethyl ester and 1,2-*bis*(*o*-aminophenoxyl)-ethyl,*N,N,N′,N′*-tetraacetic acid (BAPTA-AM) were obtained from Molecular Probe. Polycarbonate 8-μm pore membranes were purchased from Poretics Corp.

Cell culture. Human SMCs were explanted from saphenous veins harvested at peripheral arterial bypass grafting, using an explant method as described in detail previously.[6,16]

Measurement of $[Ca^{2+}]_i$. Cellular calcium can be evaluated in attached SMCs as well as SMCs in solution. We chose the latter experimental model, because unattached cells in solution are inert in that there has been no stimulus for integrin activation. Alternatively, in attached cells integrin activation may have already occurred, and may alter the effects produced by the subsequent addition of growth factors and matrix proteins.

Levels of $[Ca^{2+}]_i$ were measured with Fura-2 acetoxymethyl-ester (Fura-2), as described in detail elsewhere.[17] In brief, growth-arrested confluent SMCs were washed twice with phosphate-buffered saline solution, then gently harvested with 0.01% trypsin-EDTA. Detached cells were resuspended in DMEM and centrifuged at 1000 rpm for 5 minutes. The collected cells were then resuspended in an assay buffer (20 mmol/L of HEPES [pH 7.4], 130 mmol/L of sodium chloride, 5 mmol/L of potassium chloride, 1 mmol/L of magnesium dichloride, 1.5 mmol/L of calcium dichloride, 0.1% bovine serum albumin, and 10 mmol/L of glucose) containing 2 μmol/L of Fura-2, for 30 minutes at 37°C under 5% carbon dioxide. Cells were again washed twice, and resuspended in an assay buffer without Fura-2, for a final concentration of 1×10^6 cells per milliliter. Fluorescence was measured at 37°C with a dual-excitation wavelength spectrofluorimeter apparatus (SPEX; Fluorog-2).

Cells were placed in plastic cuvettes, and agonists were added at the beginning of each measurement to attain the final concentrations as indicated. Two excitations at 340 and 380 nm and one emission at 505 nm were recorded. The experiments were individually calibrated with the addition of 40 μmol/L of digitonin and 20 mmol/L of ethyleneglycol-*bis*-(β-aminoethylether)-*N,N,N′,N′*-tetraacetic acid (EGTA). The cytosoloic-free $[Ca^{2+}]_i$ was calculated by using the ratio 340:380 nm of the emitted fluorescence signal, as described by Grynkiewicz.[17] Values were displayed graphically with SPEX DM3000 software.

Measurement of chemotaxis. Migration assays were performed in a 48-well Boyden microchemotaxis chamber (Neuro Probe), as described.[6] Quiescent SMCs were harvested (0.01% trypsin-EDTA), and suspended (50,000 cells per well) into the upper wells of the chamber with and without agonists or inhibitors. Solutions of PDGF-AB or PDGF-BB, ECM proteins, or vehicle (0.01% bovine serum albumin and 4 mmol/L hydrochloride) in 25 μL of serum-free DMEM were added to the wells of the lower chamber of the Boyden apparatus, with upper and lower wells separated by the polycarbonate filter membranes with 8.0-μm pores. PDGF isotypes (5 ng/mL) and ECM proteins (Lm 20 μg/mL, Fn 20 μg/mL, CNI 20 μg/mL, CNIV 20 μg/mL) were dissolved in aqueous solution and acetic acid, respectively. For each experiment, appropriate vehicle served as control. Migration was allowed to occur at 37°C for 4 hours. The membrane was carefully removed, fixed with ice-cold 70% ethanol, and stained overnight with hematoxylin. The number of migrated cells on the lower side was then counted in five independent random high-power fields under 200× magnification with light microscopy. All experiments were repeated at least three times with SMC preparations from different donors. The effects

of agonists and inhibitors were expressed as a fold increase or decrease in migration ± SE compared with appropriate control sample.

Statistical analysis. Statistical comparisons were made with an unpaired Student *t* test with Statview software (SAS Institute) on a Macintosh G4 desktop computer (Apple Computer). For comparisons, $P < .05$ was considered significant. Data displayed are representative.

RESULTS

Rise in $[Ca^{2+}]_i$ is sufficient for SMC migration. To investigate whether elevation in $[Ca^{2+}]_i$ is sufficient for SMC migration we examined the effect of the calcium ionophore A23187 on $[Ca^{2+}]_i$ and SMC migration. A23187 increases $[Ca^{2+}]_i$ by inducing release of $[Ca^{2+}]_i$ stores, which in turn activate store-operated calcium channels, enabling influx of extracellular calcium.[18] SMCs were stimulated with increasing concentrations of A23187 (10^{-11}-10^{-8} mol/L). A23187 concentrations of 10^{-8} and 10^{-9} mol/L produced an increase in $[Ca^{2+}]_i$ that peaked at 25 to 45 seconds (Fig 1, *A*), and produced a significant increase in SMC chemotaxis (53% and 60% over control; $P < .05$; Fig 1, *B*). At concentrations of 10^{-10} and 10^{-11} mol/L, A23187 did not produce significant changes in $[Ca^{2+}]_i$ or SMC chemotaxis. Cell toxicity from A23187 was noted at concentrations higher than 10^{-8} mol/L.

PDGF stimulates a rise in $[Ca^{2+}]_i$. To further investigate the relationship between chemotaxis and $[Ca^{2+}]_i$ we evaluated fluctuations in $[Ca^{2+}]_i$ in response to the potent agonist of SMC migration, PDGF. Tracings that illustrate the $[Ca^{2+}]_i$ response of SMCs stimulated with 5 ng/mL of either PDGF-AB or PDGF-BB are shown in Fig 2, *A*. Both isotypes produced a rise in $[Ca^{2+}]_i$ that was observed within seconds of stimulation. Calcium levels peaked at approximately 60 seconds for PDGF-BB and at 90 seconds for PDGF-AB. We consistently found that PDGF-BB induced an early peak in $[Ca^{2+}]_i$ that was substantially greater than that for PDGF-AB. We observed a late plateau in $[Ca^{2+}]_i$ that occurred 170 to 270 seconds after PDGF stimulation that was roughly equivalent for both isotypes. In parallel experiments the effect of PDGF-AB and PDGF-BB on SMC migration was assessed (Fig 2, *B*). Both isotypes of PDGF produced a significant increase in SMC chemotaxis (PDGF-AB, 6.7; PDGF-BB, 8.8; $P < .05$). Moreover, PDGF-BB consistently induced an approximately 30% greater increase in SMC chemotaxis than PDGF-AB did. These results demonstrate that the two PDGF isotypes tested induce a pattern of $[Ca^{2+}]_i$ release and a chemotactic response that is isotype-specific.

Rise in $[Ca^{2+}]_i$ is necessary for PDGF-induced chemotaxis. We next buffered $[Ca^{2+}]_i$ with the calcium chelator BAPTA-AM. The AM derivative enables diffusion of BAPTA into cells, whereas cleavage of the AM group results in its cellular retention. Pretreatment for 30 minutes with BAPTA-AM at 50 μmol/L (Fig 3, *A*) did not alter baseline calcium levels, but eliminated the early peak and diminished the late plateau in $[Ca^{2+}]_i$ after PDGF stimulation (5 ng/mL). Chemotaxis was then measured in SMCs

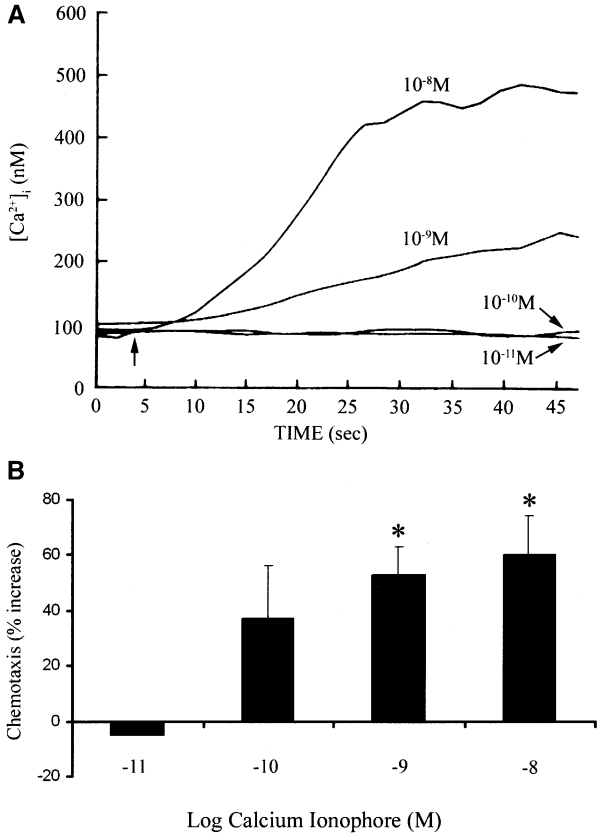

Fig 1. Effect of calcium ionophore A23187 on intracellular calcium **(A)** and smooth muscle cell (SMC) migration **(B)**. **A,** Quiescent SMCs were stimulated with A23187 (10^{-11} to 10^{-8} mol/L), and intracellular calcium was measured. *Arrow* indicates introduction of A23187. **B,** With a microchemotaxis chamber, quiescent SMCs were stimulated by adding A23187 (10^{-11} to 10^{-8} mol/L) to the bottom chamber. Chemotaxis is expressed as percent increase versus vehicle-treated control. Experiments were performed in at least three cell lines from different donors, and data shown are mean ± SE of triplicate samples. *$P < .05$, A23187 vs control.

preincubated for 30 minutes with 50 μmol/L of BAPTA-AM and then stimulated with PDGF-AB or PDGF-BB. The presence of BAPTA-AM decreased PDGF-AB–induced chemotaxis by 3.9-fold and PDGF-BB–induced chemotaxis by 5.2-fold ($P < .05$ for each; Fig 3, *B*). In parallel experiments with trypan blue exclusion, exposure of SMCs to 50 μmol/L of BAPTA-AM had no effect on cell viability (data not shown).

Late plateau in $[Ca^{2+}]_i$ is the predominant signal for PDGF-induced SMC migration. It has been established in numerous cell types that the initial peak in agonist-stimulated $[Ca^{2+}]_i$ is related to the release of intracellular stores, whereas the late plateau is related to influx of extracellular ion.[19] To verify that this pattern is similar in human saphenous vein SMCs, cells were cotreated with $NiCl_2$, which blocks the influx of extracellular calcium through

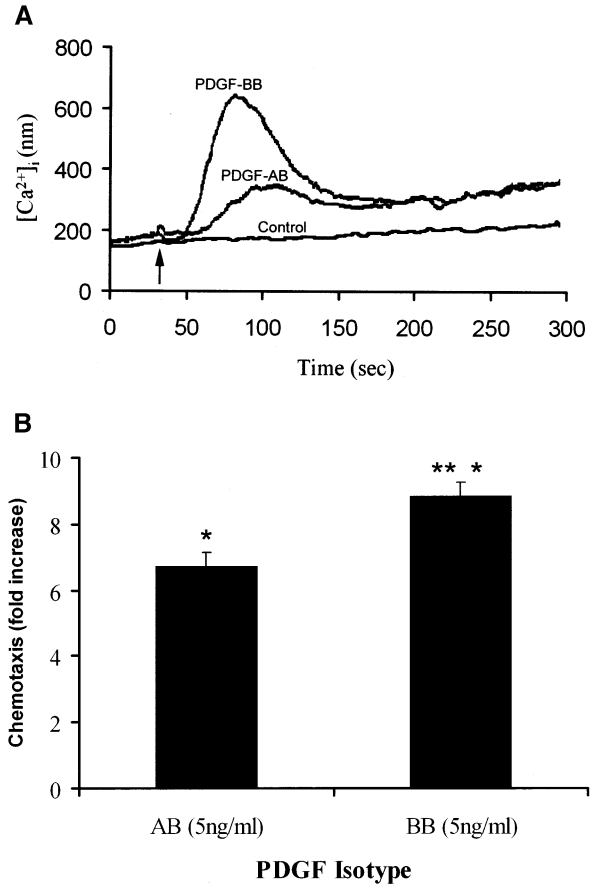

Fig 2. Effect of platelet-derived growth factor (PDGF)–AB and PDGF-BB on intracellular calcium **(A)** and smooth muscle cell (SMC) chemotaxis **(B)**. **A,** Quiescent SMCs were stimulated with PDGF-AB (5 ng/mL), PDGF-BB (5 ng/mL), and vehicle control, and intracellular calcium was measured. *Arrow* indicates introduction of agonist. **B,** With a microchemotaxis chamber, quiescent SMCs were allowed to migrate toward PDGF-AB (5 ng/mL) or PDGF-BB (5 ng/mL). Chemotaxis is expressed as fold increase vs vehicle-treated control. Experiments were performed in at least three cell lines from different donors, and data shown are mean ± SE of triplicate samples. **P* < .05, PDGF vs control. ***P* < .05, PDGF-AB vs PDGF-BB.

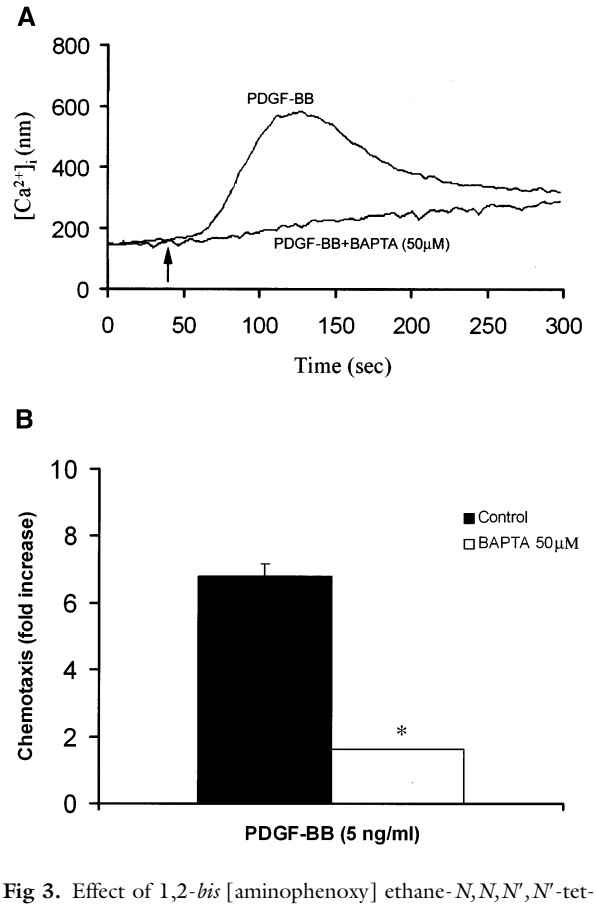

Fig 3. Effect of 1,2-*bis* [aminophenoxy] ethane-*N,N,N′,N′*-tetraacetic acid *(BAPTA)* on platelet-derived growth factor (PDGF)–BB–induced intracellular calcium **(A)** and chemotaxis **(B)**. **A,** Quiescent smooth muscle cells (SMCs) were stimulated with PDGF-BB (5 ng/mL) with or without BAPTA (30 minutes pretreatment at 50 μmol/L), and intracellular calcium was measured. *Arrow* indicates introduction of PDGF. **B,** With a microchemotaxis chamber, quiescent SMCs were allowed to migrate toward PDGF-BB (5 ng/mL) with or without BAPTA (30 minutes pretreatment at 50 μmol/L). Chemotaxis is expressed as fold increase vs vehicle-treated control. Experiments were performed in at least three cell lines from different donors, and data shown are mean ± SE of triplicate samples. **P* < .05, PDGF alone versus PDGF and BAPTA.

store-operated calcium channels and voltage-operated calcium channels, or with caffeine plus ryanodine (Caff/Ryn), which depletes sarcoplasmic reticular calcium stores. SMCs were then stimulated with PDGF-AB or PDGF-BB (5 ng/mL), and $[Ca^{2+}]_i$ was measured. Pretreatment of SMCs with $NiCl_2$ (1 μmol/L) had little or no effect on the early peak of $[Ca^{2+}]_i$ after PDGF-AB and PDGF-BB stimulation (Fig 4, *A*). However, $NiCl_2$ eliminated the late plateau for both PDGF isotypes. Pretreatment of SMCs with Caff/Ryn (20 mmol/L per 20 μmol/L for 20 minutes) resulted in loss of the early rise in $[Ca^{2+}]_i$ calcium induced by both PDGF isotypes (Fig 4, *C* and *D*). However, within 60 seconds $[Ca^{2+}]_i$ began to rise, and steadily increased to levels equivalent to that associated with the late plateau in control cells. Thus the early peak in $[Ca^{2+}]_i$ after PDGF stimulation of SMCs is related to release of intracellular calcium, and the late plateau is related to influx of extracellular calcium.

We then explored whether the PDGF-induced migration depends on the early peak or the late plateau of $[Ca^{2+}]_i$. SMCs were seeded onto the membranes of a microchemotaxis chamber and allowed to attach for 2 hours. Cells were then co-treated with $NiCl_2$ (1 μmol/L) or pretreated with Caff/Ryn (20 mmol/L per 20 μmol/L) for 20 minutes before introduction of PDGF. In SMCs treated with $NiCl_2$ there was, respectively, a 4.6-fold and 5.5-fold reduction ($P < .05$) in PDGF-AB–induced and

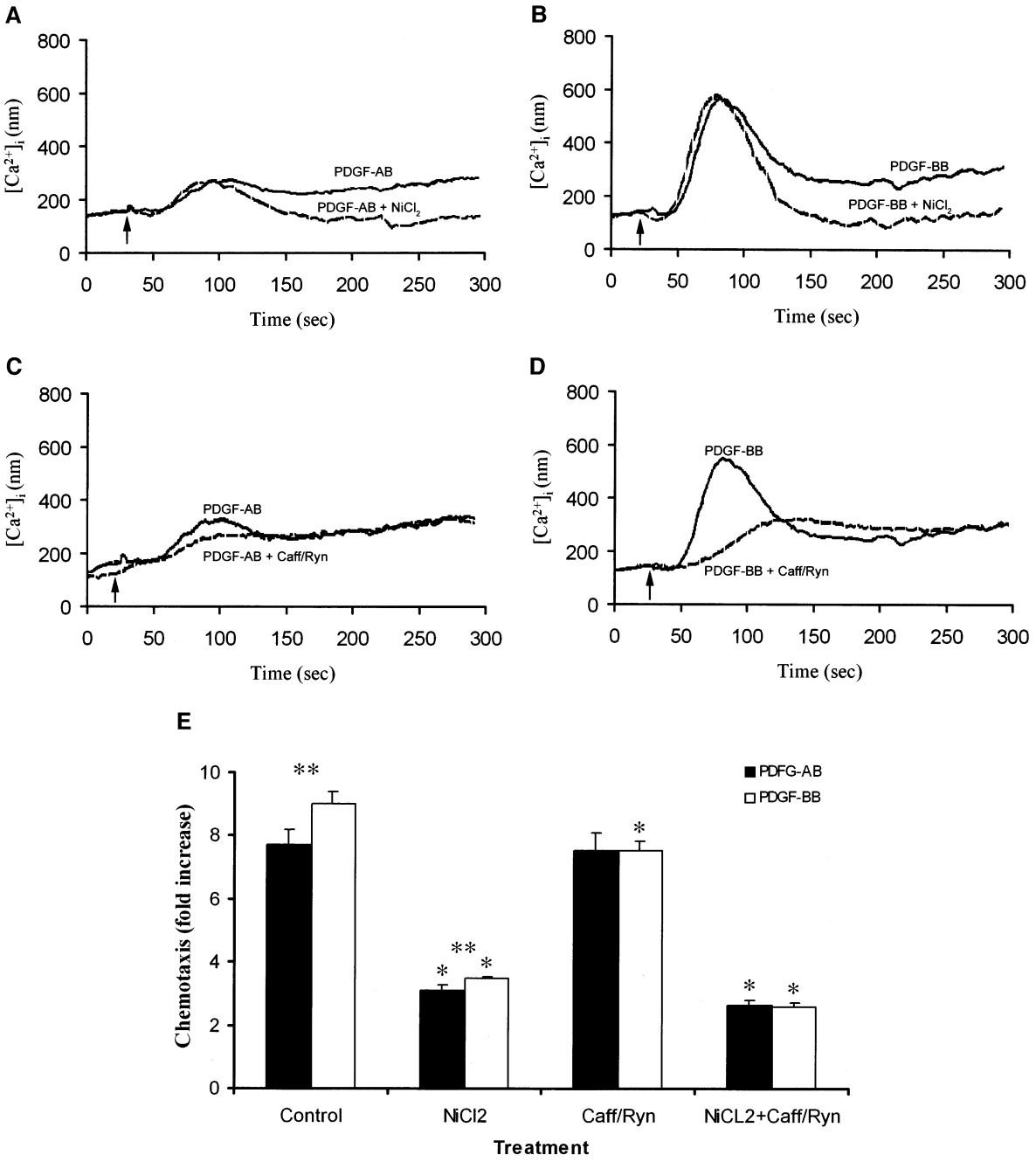

Fig 4. Effects of nickel chloride *($NiCl_2$)* and caffeine plus ryanodine *(Caff/Ryn)* on platelet-derived growth factor (PDGF)–AB–induced and PDGF-BB–induced intracellular calcium **(A-D)** and chemotaxis **(E)**. Quiescent SMCs were stimulated with PDGF-AB (5 ng/mL; **A**) and PDGF-BB (5 ng/mL; **B**), with or without $NiCl_2$ (1 μmol/L), and intracellular calcium was measured. *Arrow* indicates introduction of PDGF. Quiescent SMCs were stimulated with PDGF-AB (5 ng/ml; **C**) and PDGF-BB (5 ng/mL; **D**), with or without Caff/Ryn pretreatment (20 mmol/L per 20 μmol/L for 20 minutes), and $[Ca^{2+}]_i$ was measured. *Arrow* indicates introduction of PDGF. **E,** With a microchemotaxis chamber, quiescent SMCs were allowed to migrate toward PDGF-AB (5 ng/mL) or PDGF-BB (5 ng/mL), with or without $NiCl_2$ (1 μmol/L) or Caff/Ryn pretreatment (20 mmol/L per 20 μmol/L for 20 minutes). Chemotaxis is expressed as fold increase vs vehicle-treated control. Experiments were performed in at least three cell lines from different donors, and data shown are mean ± SE of triplicate samples. * $P < .05$, PDGF plus $NiCl_2$ or PDGF plus Caff/Ryn or PDGF plus Caff/Ryn and $NiCl_2$ vs PDGF alone. ** $P < .05$, PDGF-AB vs PDGF-BB.

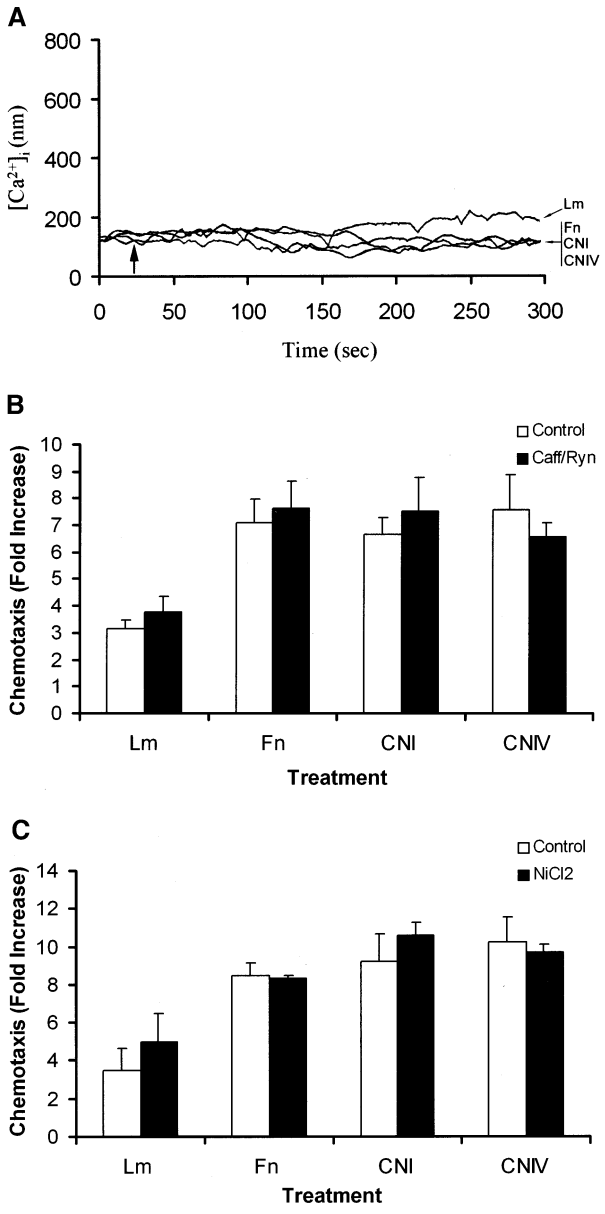

Fig 5. A, Quiescent smooth muscle cells (SMCs) were stimulated with the extracellular matrix (ECM) proteins laminin (*Lm*, 20 μg/mL), fibronectin (*Fn*, 20 μg/mL), collagen type I (*CNI*, 20 μg/mL), or collagen type IV (*CNIV*, 20 μg/mL), and intracellular calcium was measured. *Arrow* indicates introduction of ECM proteins. **B,** With a microchemotaxis chamber, quiescent SMCs were allowed to migrate toward the same four soluble ECM proteins in the presence or absence of $NiCl_2$ (1 μmol/L; **B**) or Caff/Ryn (20 mmol/L per 20 μmol/L for 20 minutes; **C**). Chemotaxis is expressed as fold increase vs control. Experiments shown were performed in at least three cell lines from different donors, and data shown are mean ± SE of triplicate samples. There were no significant differences between ECM protein, and ECM protein and Caff/Ryn.

PDGF-BB–induced chemotaxis (Fig 4, *E*). In contrast, cells pretreated with Caff/Ryn demonstrated no change in PDGF-AB–induced chemotaxis, and a 1.4-fold decrease in chemotaxis in response to PDGF-BB ($P < .05$). Pretreatment of SMCs with both $NiCl_2$ and Caff/Ryn produced, respectively, a 5.1-fold and 6.4-fold reduction ($P < .05$) in chemotaxis for PDGF-AB and PDGF-BB. These reductions were similar to those observed with $NiCl_2$ alone.

ECM-induced SMC chemotaxis is mediated by an alternate, calcium-independent signaling pathway. We have previously shown that ECM proteins such as Lm, Fn, CNI, and CNIV induce chemotaxis of SMCs.[6] We therefore postulated that $[Ca^{2+}]_i$ might have a similarly important role in chemotaxis of SMCs in response to ECM. Thus we evaluated whether ECM proteins could produce a rise in $[Ca^{2+}]_i$ in human SMCs. SMCs were loaded with the fluorescent indicator Fura-2, then exposed to soluble ECM proteins (Lm, 20 μg/mL; Fn, 20 μg/mL; CNI, 20 μg/mL, or CNIV, 20 μg/mL). $[Ca^{2+}]_i$ transients were not observed after stimulation with the various ECM proteins despite observing calcium levels for up to 4 minutes (Fig 5, *A*).

To confirm that intracellular calcium transients are not necessary for ECM-induced chemotaxis we stimulated SMCs with ECM proteins in the presence of $NiCl_2$ (1 μmol/L) or after pretreatment with Caff/Ryn (20 mmol/L per 20 μmol/L for 20 minutes). ECM proteins induced a significant increase in SMC migration, as anticipated (Fig 5, *B*). However, treatment of SMCs with $NiCl_2$ did not significantly alter SMC migration in response to Fn, Lm, CNI, and CNIV (Fig 5, *B*). Thus, contrary to our findings with PDGF, ECM-induced chemotaxis does not require the influx of extracellular calcium. To evaluate whether ECM protein–induced chemotaxis requires release of intracellular calcium stores we performed similar studies with Caff/Ryn. Pretreatment with Caff/Ryn also did not alter the SMC response to Fn, Lm, CNI, or CNIV (Fig 5, *C*). These results indicate that vascular SMC chemotaxis in response to the ECM proteins Fn, Lm, CNI, and CNIV is independent of calcium transients.

DISCUSSION

SMC migration is critical to development of intimal hyperplasia. In previous studies we found soluble ECM proteins to be potent stimuli of SMC migration; their stimulus is equivalent to that of PDGF.[6] Moreover, PDGF and ECM proteins interact synergistically to promote SMC chemotaxis.[7] One method by which intimal thickening might be controlled is through inhibition of the signaling events necessary for SMC migration. Consequently we explored the role of calcium in migration produced by both growth factors and extracellular matrix.

Calcium ionophore produced a rapid rise in $[Ca^{2+}]_i$ that mimicked the early peak observed when SMCs were stimulated with growth factors such as PDGF. A late plateau in $[Ca^{2+}]_i$ was also observed in response to ionophore, but the intensity was substantially less than the plateau observed with PDGF. The changes in $[Ca^{2+}]_i$ produced by ionophore were sufficient to produce SMC chemotaxis, albeit the response was small. Thus a rise in $[Ca^{2+}]_i$, independent of other stimuli, is sufficient to produce SMC migration. Using BAPTA to sequester $[Ca^{2+}]_i$, we found

that migration of human saphenous vein SMCs in response to PDGF is calcium-dependent. The effect of eliminating calcium fluctuations on PDGF-induced migration was profound, with levels of chemotaxis returning near baseline. These data suggest a critical role for calcium in the migration of vascular SMCs in response to PDGF.

It is well established in multiple cell types that PDGF produces a rise in $[Ca^{2+}]_i$. Binding of PDGF to its receptor results in rapid production of inositol 1,4,5-triphosphate, which stimulates release of calcium stores from the sarcoplasmic reticulum.[19] This early peak rise in $[Ca^{2+}]_i$ is consistently followed by a prolonged plateau in $[Ca^{2+}]_i$ related to influx of extracellular calcium through membrane channels.[20] Although we observed a similar temporal pattern in human vascular SMCs stimulated with PDGF, whether the early peak or the late plateau in $[Ca^{2+}]_i$ is responsible for PDGF-induced cellular migration has not been explored. To determine the relationship between temporal elevations in $[Ca^{2+}]_i$ and SMC migration we used inhibitors that selectively block the early peak and the late plateau. Blocking the release of calcium from the sarcoplasmic reticulum and thus the initial rise in $[Ca^{2+}]_i$ did not substantially influence migration. Alternatively, elimination of the later plateau in $[Ca^{2+}]_i$ markedly inhibited SMC chemotaxis for both isotypes of PDGF. Thus the chemotactic response of PDGF appears to be largely related to influx of extracellular calcium.

The timing of calcium release and its importance have been explored in relation to SMC functions other than migration. Using $NiCl_2$ and Caff/Ryn, Kobayashi et al[21] found that neither early nor late calcium transients were necessary for SMC mitosis or division. SMC contraction appears to be related to both release of intracellular calcium stores and influx of extracellular calcium, depending on the stimulus. Influx of extracellular calcium is required for arterial vasoconstriction resulting from increased intraluminal pressure.[22] In contrast, α-adrenergic activation leading to vascular SMC contraction is mediated primarily by release of intracellular calcium stores.[23] These and other studies demonstrate that the timing of calcium release and the source of calcium are critically important in determining the effect of calcium on cellular function.

On the basis of our findings with PDGF, we postulated that ECM protein–induced SMC chemotaxis would also depend on calcium. However, we were not able to observe a rise in $[Ca^{2+}]_i$ in human saphenous vein SMCs stimulated with ECM proteins. Moreover, ECM-induced SMC chemotaxis was unaffected by treatment with either Caff/Ryn or $NiCl_2$. In contrast to our findings, numerous investigators have shown that migration in response to ECM in most cell types depends on a rise in $[Ca^{2+}]_i$. There are some exceptions. Leavesley et al[24] demonstrated that endothelial cell migration in response to collagen can occur in the absence of $[Ca^{2+}]_i$. Migration of endothelial cells on collagen type I is mediated by the α2β1 integrin, which suggests that α2β1 can stimulate migration independent of calcium.[24] Conversely, vitronectin-mediated endothelial cell migration, which is mediated by the αVβ3 integrin, depends on $[Ca^{2+}]_i$ fluctuations.[24] These observations parallel our findings with vascular SMCs. In previous studies with saphenous vein SMCs we showed that blocking antibodies to α2β1 inhibit chemotaxis in response to these same four matrix proteins, whereas antibodies to αVβ3 do not.[25] Moreover, we now show that SMC migration in response to CNI, CNIV, Fn, and Lm is calcium-independent. Thus in human saphenous vein SMCs α2β1 also appears to facilitate migration in response to various matrix proteins via a pathway that is independent of calcium. The ability of α2β1 to stimulate migration independent of calcium seems universal. Hodgson and Dong[26] observed in human melanoma cells that a rise in $[Ca^{2+}]_i$ was neither necessary nor sufficient for α2β1-mediated cell migration on collagen type IV. Of interest, human aortic SMCs seem to behave differently. Bilato et al[27] found that migration of rat aortic SMCs on Fn is mediated by αVβ3 and is calcium-dependent.

It was predicted that calcium channel blockers would prevent recurrent stenosis after coronary angioplasty. However, several large prospective trials have revealed little or no advantage to these agents in prevention of recurrent arterial occlusive disease.[28,29] It is interesting that our data predict success of calcium channel blockers as inhibitors of SMC migration in response to PDGF but failure of these agents to block the equally potent stimulus for migration provided by ECM. Migration is admittedly only one component of the hyperplastic process, and the complete explanation for the failure of calcium channel blockers in treatment of recurrent stenosis may be much more complex.

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