Enhancement of Migration by Protein Kinase Cα and Inhibition of Proliferation and Cell Cycle Progression by Protein Kinase Cδ in Capillary Endothelial Cells*

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Activation of protein kinase C (PKC) induces angiogenesis, migration, and proliferation of endothelial cells (EC), but can also prevent growth factor-induced EC proliferation. To determine whether these disparate effects are mediated by substrates of individual PKC isoenzymes, PKCα and PKCδ were overexpressed in rat microvascular EC. Basal and stimulated migration were enhanced in PKCα EC compared with either PKCδ or control EC. Serum-induced growth of PKCδ EC was decreased, while that of PKCα cells was similar to control EC. Phorbol ester markedly inhibited PKCα EC growth but enhanced growth of PKCs and control EC. To determine possible causes for this altered proliferation, the effect of PKCδ on adhesion, mitogen-activated protein kinase activity, and cell cycle progression was measured. Adherence of PKCδ EC to vitronectin was significantly enhanced. Serum-induced extracellular signal-regulated kinase-2 activity was increased equally in both PKCα and PKCδ EC above that of control, while extracellular signal-regulated kinase-1 activity was similar in all EC. Cell cycle analysis suggested that PKCδ EC entered S phase inappropriately and were delayed in passage through S phase. Thus, PKCα may mediate some proangiogenic effects of PKC activation; conversely, PKCδ may direct antiangiogenic aspects of overall PKC activation, including slowing of the cell cycle progression.

The formation of new blood vessels and the repair of those damaged by disease or injury depend upon endothelial migration and proliferation (1, 2). Several external agents that promote or inhibit proliferation and migration have been identified (3, 4), but the intracellular messengers that mediate these processes are less clear.

Activation of the serine-threonine kinase protein kinase C (PKC)† by phorbol esters induces migration, proliferation (5), and tube formation of cultured endothelial cells (6, 7) and causes angiogenesis in vivo (7–9). In addition, chemical inhibitors of PKC or the down-regulation of PKC by prolonged treatment with phorbol esters abrogates the proliferative effects induced by growth factors and mitogens (10, 11) and also enhances endothelial permeability (12, 13) and alters the expression level of several fibrinolytic enzymes and their inhibitors (14). In contrast, treatment of endothelial cells with direct activators of PKC alters some responses that are usually associated with stimulation by physiologic agonists (15) and, under some conditions, can prevent growth factor-induced proliferation (16). This apparent paradox might be explained by the fact that the PKC family is composed of related but structurally distinct isoenzymes, each a product of separate genes and with discrete cofactor requirements, substrate specificity, and tissue distribution (16–18). Since phorbol esters activate multiple isoenzymes of PKC, the possibility is raised that each PKC isoenzyme may selectively mediate separate, and perhaps opposing, effects within stimulated endothelial cells.

Preliminary studies in our laboratory revealed that rat capillary endothelial cells expressed several isoenzymes of PKC, including PKCα, -δ, -η, -ζ, and -ζ. Of these isoenzymes, previous investigations have found that overexpression of PKCα or PKCδ in various cultured cells could affect their proliferation (19–21). Overexpression of PKCα in fibroblasts promoted their proliferation, while proliferation was inhibited in human breast cancer cells and other cell lines (20, 22–24); similar alterations of cell growth have been observed in cells overexpressing PKCδ (20, 21, 25). Thus, the possibility exists that activation of either of these isoenzymes mediates the inhibitory component of PKC activation on endothelial growth, while the other promotes one or more processes essential to endothelial repair and angiogenesis. Such an effect would be presumably mediated by isoenzyme-specific substrates, of which a few have been identified (e.g. elongation factor eEF-1α (26)). In the present study, we began by testing the effect of overexpression of PKCα and PKCδ on endothelial migration and proliferation, both of which are essential processes for angiogenesis and wound healing. When initial experiments revealed an inhibitory effect of PKCδ on endothelial proliferation, we then examined potential underlying causes.

**EXPERIMENTAL PROCEDURES**

Construction of Rat Fat Pad Epididymal Endothelial Cell (RFPEC) Cell Lines Overexpressing PKCα and PKCδ—The RFPEC were a generous gift from R. D. Rosenberg (MIT) (27, 28) and were propagated in M199 medium (Life Technologies, Inc.) supplemented with 2 mM l-

PMA, phorbol 12-myristate 13-acetate; HGF, hepatocyte growth factor.
glutamine, penicillin (10 units ml\(^{-1}\)), streptomycin (10 units ml\(^{-1}\)), and amphotericin B (250 ng ml\(^{-1}\)). To obtain the RFPBEC that stably express vector (control), PKCa, or PKC\(\delta\), pcDNA-Neo (Invitrogen, Inc.), pcDNA-hPKC\(a\), or pcDNA-hPKC\(\delta\) constructs, respectively, were transfected into early passage RFPBEC cells by the calcium phosphate precipitation method. Following selection in G418, a number of vector-transfected, PKCa-transfected, and PKC\(\delta\)-transfected clones of endothelial cells (designated as control EC, PKCa EC, and PKC\(\delta\) EC) were isolated and expanded, and the mRNA was examined by Northern blot analysis for expression of the respective transscripts.

**Determination of Protein Kinase C Activity—**Cells were removed from subconfluent cultures of stably transfected EC chamber. The cell suspension was then added to the upper wells of the chamber at a density of 5 \times 10^5 cells/well. Chemotaxis was assayed over 4 h at 37 \degree C in a CO\(_2\) incubator, under both unstimulated (basal) and stimulated (25 ng of hepatocyte growth factor/scatter factor per ml of medium) conditions. The membrane was removed from the chamber, fixed in 70% ethanol for 20 min, and stained in hematoxylin overnight. The upper surface of the stained membrane was scraped using a cotton swab, leaving only the cells that migrated to the undersurface. Migration was assessed by counting the number of cells on the lower surface of the membrane at \(x \times 200\) magnification by light microscopy.

**Proliferation Assay—**Endothelial cells were seeded at equivalent densities in six-well culture dishes and allowed to adhere overnight in complete medium. Following wash with PBS, cells were incubated for 24 h in M199 without serum. The cells were subsequently stimulated with 1 mg of purified human vitronectin resuspended in Ca\(^{2+}\), Mg\(^{2+}\)-free PBS (pH 7.4) for 1 h at 37 \degree C. The plates were rinsed with the same buffer, coated with 1% heat-denatured BSA in the same buffer, and incubated at room temperature for 30–60 min. Actively growing (50–80% confluent) cultures of RFPBEC were removed from the culture plate with trypsin, washed, and resuspended in M199 supplemented with 0.5% BSA. Cells were plated in each matrix-coated well at 2.5 \times 10^5 cells/well and incubated at 37 \degree C for 60–90 min. The unadhered cells were removed, and the wells were gently washed. Adherent cells were detected by incubating in the presence of 6 mg ml\(^{-1}\) p-nitrophenylphosphate (Sigma) in 50 mM sodium acetate (pH 5), 1% Triton X-100, 10% wash in 1% Triton X-100, 10% BSA, 0.1% sodium deoxycholate, and the absorbance was determined at 405 nm using an enzyme-linked immunosorbent assay plate reader.

**Flow Cytometry—**The cells were seeded, synchronized by serum deprivation for 72 h, and stimulated as described for the proliferation assay. DNA flow cytometric analysis of stably overexpressing EC cells was performed using a technique described by Tennenbaum et al. (30). The experiments were performed in a four-color flow cytometer capable of acquiring with 0.0035% trypsin in sample buffer (3.4 mM sodium citrate, 0.1% Nonidet P-40, 1.5 mM spermine HCl, 0.5 mM Tris-Cl, pH 7.6). The reaction was stopped by the addition of 0.05% trypsin inhibitor, 0.01% ribonuclease A in sample buffer. The cells were subsequently treated with ice-cold 0.042% propidium iodide, 0.116% spermine HCl in sample buffer. The cells were kept on ice and in aluminum foil until analyzed. Flow cytometry was performed on a FACStar plus flow cytometer at an excitation of 488-nm wavelength and 630DF22 emission, and the data were analyzed using the Verity MODFIT software.

Stably transfected control, PKCa, and PKC\(\delta\) EC were removed with trypsin from the culture plate and resuspended in PBS at 5 \times 10^5 cells ml\(^{-1}\) for the flow cytometric analysis of the surface expression of \(\beta_3\) or \(\beta_6\) integrins. Fifty-microliter aliquots of the cell suspensions were incubated in the presence of optimal concentrations of the primary antibody (1 mg of rabbit anti-rat \(\beta_3\) IgG fluorescein isothiocyanate-conjugated (Pharmingen, Inc.) or 0.5 \mu l of rabbit anti-human \(\beta_6\) polyclonal antibody (Chemicon, Inc.) per 50 ml reaction) at room temperature for 15 min. The cells were pelleted at 1000 \times g for 5 min at 4 \degree C. When necessary, the cells were resuspended in 50 \mu l of PBS and incubated with appropriate concentrations of the secondary antibody (1:20 rabbit IgG fluorescein isothiocyanate-conjugated (Jackson Immunochemicals, Inc.)) for 15 min at room temperature. The cells were washed twice with PBS and resuspended in 200 \mu l of PBS. Fluorescence bound to cells was detected with a FACStar plus flow cytometer set at a 488-nm excitation wavelength and 530DF30 emission.
were rendered quiescent for 24 h prior to the assay. The cells were stimulated with M199 supplemented with 15% FBS or 1 μM PMA for the indicated times. The cells were then harvested by removing the medium, washing once with ice-cold PBS, and incubating in radioimmunoprecipitation buffer (50 mM Heps, pH 7.4, 150 mM NaCl, 5 mM EGTA, 5 mM EDTA, 20 mM NaF, 20 mM sodium pyrophosphate, 1% Triton X-100, 1 mM sodium orthovanadate, 1 mM phenylmethylsulfonyl fluoride) on a rocking incubator at 4°C for 30 min. The lysed cells were scraped from the culture dish and transferred to a microcentrifuge tube. Large cellular debris was removed from the protein suspension by centrifugation at 15,800 × g for 5 min at 4°C. The cleared protein extract was then transferred to a fresh microcentrifuge tube, and total protein concentration was determined by means of the bicinchoninic acid assay (Pierce). MAP kinase was immunoprecipitated using specific antibodies (Santa Cruz Biotechnology) from extract containing equal amounts of protein. After the sample volumes were adjusted with radioimmunoprecipitation buffer, the ERK-1 or ERK-2 antibody (1 μg of antibody/250 μl total protein) was added. The extracts were incubated on a rocking incubator at 4°C overnight. The immune complexes were pelleted with protein A-agarose (Life Technologies, Inc.), washed three times in radioimmune precipitation buffer, and suspended in 20 μl of kinase buffer (10 mM Tris-Cl, pH 7.5, 150 mM NaCl, 10 mM MgCl2). ERK-2 activity was assayed by incubating 20 μl of each sample with 20 μl of the reaction mixture (8 μg of myelin basic protein (Sigma), 0.5 μCi of [γ-32P]ATP (specific activity 3000 mCi/mmol) (DuPont NEN), and 10 μM ATP) for 30 min at 25°C. The reaction was quenched with 15 μl of 4 × Laemmli buffer. The phosphorylated myelin basic protein was then resolved on a 15% SDS-polyacrylamide separating gel with a 4% polyacrylamide stacking gel and visualized by autoradiography.

**RESULTS**

**Isolation and Characterization of Overexpressing Cell Lines**—To investigate the role of PKCα and PKCδ isoenzymes in relation to angiogenesis, stably transfected microvascular RFPEC were established that overexpressed the eukaryotic expression vector pcDNA-Neo containing the complete cDNA sequence of PKC isozyme PKCα or PKCδ or, as a control, the vector expression without an inserted gene (PKCα EC, PKCδ EC, and control EC, respectively). The stably expressing RFPEC cell lines were selected by neomycin resistance and screened by Northern blot analysis for gene expression (Fig. 1).

The stably transfected RFPEC displayed the cobblestone morphology typical of endothelial cells and were not visibly altered by transfection or overexpression. Immunoblot analysis demonstrated increased protein production of the corresponding PKC isoenzymes. The enzymatic activity of PKC was determined in several cell lines. Following this initial analysis, two clones of each type (PKCα EC and PKCδ EC) were chosen for further study on the basis of similar levels of total kinase activity. Table I summarizes the PKC activity of the control and the two selected PKCα and PKCδ EC lines, revealing total PKC activity that was increased in both the cytosolic and cytoskeletal fractions and was comparable between PKCα and PKCδ EC.

To ensure that overexpression of PKCα and PKCδ isoenzymes in the endothelial cells did not cause abnormal subcellular localization, we assessed the intracellular location of these PKC isoenzymes by immunofluorescence in both quiescent and PMA-treated EC. Experiments in PMA-treated EC were performed because activation of some PKC isoenzymes is associated with their redistribution into distinct subcellular locations (31). In quiescent control EC, PKCα could be detected primarily within the cytoplasm and nucleus (Fig. 2A). Staining of these cells with antibodies directed against histone proteins or with a fluorescently tagged ceramide confirmed the nuclei and Golgi apparatus structures (Fig. 2, I and J), respectively. Following a 10-min incubation with PMA, PKCα could still be seen in the nucleus, but also at the periphery of the cell along the plasma membrane (Fig. 2B). Interestingly, PKCα translocated primarily to regions of the plasma membrane in which there was cell-cell contact. A similar cellular distribution of PKCα was noted in both the PKCα EC (Fig. 2, C and D) and in the PKCδ EC (data not shown). Immunofluorescent staining for PKCδ demonstrated the nuclear and cytosolic location for this isozyme in serum-starved control EC (Fig. 2E). PKCδ redistributed to the plasma membrane and the nuclear membrane upon activation with PMA (Fig. 2F). A similar pattern of staining for PKCδ was noted in the PKCα (data not shown) and PKCδ (Fig. 2, G and H) EC. Thus, constitutive overexpression of PKCα or PKCδ did not affect normal cellular localization in either stimulated or quiescent endothelial cells, although enzymatic activity was increased.

**Effect of PKC Isoenzymes on Endothelial Cell Migration**—To determine the role of PKCα and δ in endothelial cell migration, the respective stably transfected cell lines were seeded in a microchemotaxis chamber, and the number of endothelial cells that migrated through the polycarbonate membrane was determined as described under “Experimental Procedures.” When hepatocyte growth factor (HGF or scatter factor), a powerful stimulus for migration and angiogenesis (32, 33), was utilized as the agonist, PKCα EC traversed the membrane at a significantly greater rate than did PKCδ or control EC (Fig. 3), suggesting a migratory response mediated by PKCα to this stimulus. The basal rate of migration (i.e. that occurring in the absence of any chemotactic agent) of PKCα was also consistently greater than that of the control EC or PKCδ EC (Fig. 3), further implicating a specific role for PKCα in enhancing endothelial cell migration. Thus, both basal and agonist-stimulated endothelial migration differed between PKCα and PKCδ EC, and PKCα EC migration was enhanced from the response seen in control EC.

**Effect of PKCα and PKCδ on Endothelial Cell Proliferation**—Stimulation of quiescent PKCα EC with low (1%) concentrations of serum induced a growth rate similar to that of the vector control EC (Fig. 4A). In contrast, PKCδ EC exhibited much less proliferation in response to serum stimulation than did either the control or PKCα EC. Proliferation of the control EC and PKCα EC in response to PMA was mildly enhanced.
PKC would lessen adhesion to extracellular matrices, prevent mitogen-activated protein kinase (MAP kinase) activation, or alter cell cycle progression in EC. To determine whether integrin-mediated endothelial adhesion, an event that is required for endothelial proliferation and angiogenesis (29, 34, 35), was lessened by PKCδ, we examined the adhesion of subconfluent cultures of control, PKCα, and PKCδ EC seeded on vitronectin-coated plates. Rather than being diminished, the ability of the PKCδ EC to adhere to the extracellular matrix was significantly enhanced above that seen with the control EC (Fig. 5), with a mean increase in adherence of 32.4% (p < 0.005). In contrast, increased PKCδ expression did not significantly alter the ability of the endothelial cells to adhere to vitronectin. Preincubation of these cells with a synthetic peptide, GRGDSP, which corresponds to the vitronectin protein sequence that directly interacts with the integrin receptor binding domain, abolished adherence of these cells, thus demonstrating that the base line adherence of the cells, plus that enhanced in PKCδ EC, was specific for the integrin receptors. To determine whether the enhanced adhesion resulted from increased expression of integrin receptors on the cell surface, we analyzed the cellular surface expression level of αvβ3 and αvβ6 by immunofluorescence using flow cytometry. Neither the overall cellular surface expression level of β3 nor that of β6, was significantly enhanced in PKCδ EC as compared with PKCα or control EC (data not shown). Thus, enhanced adhesion in PKCδ EC most probably resulted from increased affinity modulation of the integrin receptors. These results, therefore, demonstrate that the reduction in cell growth in PKCδ EC did not result from impaired adhesion to extracellular matrices.

Serum-induced ERK-2 Activation in PKCα and PKCδ EC—We next tested the possibility that impaired PKCδ EC growth resulted from impaired activation of one of the MAP kinases, ERK-1 or ERK-2, that are known to be activated following overall PKC activation (36, 37) in EC. Serum stimulation of vector (control) EC that had been rendered quiescent demonstrated a rapid increase in ERK-2 activity by 10 min, with a gradual diminution of the kinase activity by 2–4 h after stimulation (Fig. 6, A and B). ERK-2 activity also increased within 10 min following serum stimulation of the PKCα and PKCδ EC; however, the activity was enhanced above control and remained elevated above the basal kinase activity even at 4 h following stimulation. Phorbol ester treatment of the stably transfected cells resulted in similar levels of ERK-2

Effects of PKCα and PKCδ Overexpression on Endothelial Cell Adhesion—In order to better understand the cause of the decreased proliferation in PKCδ EC, we next asked whether...
however, there were no noticeable differences between the control, PKCo, and PKCδ EC (data not shown). Thus, PKCδ and PKCo appeared to be equally effective in activating ERK-2α, and thus the decrease in proliferation in PKCδ EC appeared to result from a mechanism independent of ERK-1 or ERK-2 activity.

**Effect of PKCδ and PKCo on EC Cell Cycle Progression**—To determine if an alteration in cell cycle progression might explain the decrease in cell growth in PKCδ EC, cell cycle analysis was performed on serum-deprived and stimulated PKCδ, PKCo, and control EC. After 72 h of serum starvation, 19.8 ± 4.2% of control EC and 17.1 ± 4.0% PKCδ EC were in S phase, with 74.2 ± 5.5% and 77.3 ± 0.9% in Gp/G1, respectively (Fig. 7A). In contrast, 26.3 ± 2.2% of the PKCo cells were in S phase, with 66.5 ± 1.9% in Gp/G1. These data indicate that an abnormally high percentage of PKCδ EC entered S phase inappropriately, i.e., under conditions of serum deprivation. Stimulation with serum caused control EC and PKCo EC to reenter the cell cycle normally (Fig. 7, B and C). PKCo EC, on the other hand, after an initial increase in the percentage of cells in Gp/M phase at 6 h, followed by an increase in cells in Gp/G1 at 12 h after serum, returned to a very high percentage of cells in S phase up to 60 h (Fig. 7D). The prolonged time that a high percentage of PKCδ EC could be found in S phase suggested that these cells required an abnormal amount of time to complete S phase.

**DISCUSSION**

The two major findings of this study are that overexpression of two different PKC isoenzymes normally expressed in microvascular endothelial cells exert distinct effects on endothelial proliferation, migration, and adhesion to extracellular matrix and that PKCδ-mediated inhibition of endothelial growth results from a defect in S phase of the endothelial cell cycle. The observation that overexpression of PKCδ, but not PKCo, prevents proliferation of microvascular endothelial cells, while PKCo enhances their migration in response to HGF (scatter factor), suggests that these isoenzymes phosphorylate different substrates in these cells with different physiologic effects. The disparity between the effects of overexpression of the two isoenzymes is heightened by PKC-activating phorbol esters in that treatment of PKCo EC exerts a mitogenic effect similar to that in control cells, while PKCδ EC were even more strongly inhibited by similar treatment. Thus, these data suggest that activation of each of these isoenzymes by angiogenic stimuli, such as HGF or those contained in serum, may mediate distinct aspects of several processes that are required for vascular
repair and angiogenesis. Stimulation of endothelium with phorbol esters, which activate both PKCα and PKCδ, as well as several other isoenzymes expressed in endothelium (5, 38), has been noted to have both stimulatory and inhibitory effects on endothelial proliferation and angiogenesis (5, 10, 39, 40) that can be temporally dispersed within the same cells (41). The results from this study suggest that PKCδ might mediate those aspects of PKC activation that are inhibitory for endothelial repair and angiogenesis, while an important component of the proangiogenic effect, endothelial migration, might be mediated by PKCα.

Repair of small defects in endothelium are accomplished largely by endothelial migration, rather than proliferation (3, 42). Migration is an important component of angiogenesis as well (3). The present study's finding that endothelial migration is enhanced in PKCα EC might result from enhancement of cytoskeletal reorganization in response to stimuli, which is a necessary component of cell locomotion; overall PKC stimulation has been associated with promotion of cytoskeletal reorganization of endothelial cells (3, 43). Our results suggest that PKCα, but not PKCδ, may be at least one mediator of migration response to HGF (scatter factor), a powerful angiogenic agent that is present along with its receptor in a substantial amount in the vasculature (4, 32, 44, 45).

We considered several possible explanations for the PKCδ-mediated inhibition of endothelial growth, including a reduction in endothelial adhesion to matrix, a failure to activate downstream mediators such as ERK-1 or ERK-2, and a defect in progression of endothelial cells through the cell cycle. Of these explanations, only the latter appears to be the case. Regarding adhesion, both endothelial cell growth and migration are thought to require attachment of the cell to matrix via its integrin surface receptors (42, 46, 47). Blocking the vitronectin integrin receptors αvβ3 or α5β1 inhibits neovascularization in the cornea or chick chorioallantoic membrane models (35), suggesting the importance of these two integrins for endothelial cell proliferation. In this study, microvascular endothelial cells in which PKCδ was overexpressed demonstrated markedly enhanced adhesion to vitronectin or fibrinogen matrices, and thus a decrease in integrin mediated adhesion was not the cause of the decrease in proliferation of PKCδ EC. This enhancement of adhesion could result from either a PKC-mediated alteration of the number of these receptors or an increase in avidity by either a direct effect on integrin conformation (so-called “inside-out” signaling) (48) or as an amplification step following cell adhesion (“outside-in”) that prevents detachment (48). Overall PKC activation has been linked with promotion of integrin avidity for soluble fibrinogen and solid matrices in several cell types (49, 50). Neither PKCα EC nor PKCδ EC demonstrated increased expression of these receptors when compared with control; thus, a PKCδ-mediated effect on affinity of these integrins for vitronectin is a likely explanation for our results.

A cascade of signaling events merging at the MAP kinase family of proteins, ERK-1 and ERK-2, is involved in many of the intracellular signaling pathways that lead to endothelial cell growth, migration, and adhesion (51). Activation of overall PKC leads to activation of MAP kinase; PKCα, at least, has been shown to phosphorylate Raf kinase (40), an upstream mediator of MAP kinase (36). In our studies, both PKCα and PKCδ enhance ERK-1 and ERK-2 kinase activity with a resultant prolongation of ERK-2 activity in stably transfected endothelial cells. The pathway by which PKCδ blocks proliferation and cell cycle progression, however, must be distal or parallel to that leading to ERK-2 or ERK-1. In addition, our results suggest that effective activation of ERK-2 activity by PKCδ is not sufficient for endothelial cell proliferation. This finding bolsters those of Hirai et al. (25), who found that PKCδ inhibited growth of Ras-transformed NIH 3T3 cells despite activating AP-1, a component of the signaling pathway downstream from MAP kinase.

Inhibition of endothelial cell proliferation by PKCδ appears to result from a specific defect in endothelial cell cycle progression, in which the cells enter S phase inappropriately and require additional time to complete this phase. Because of the distal nature of this defect and the intranuclear location of PKCδ, it seems likely that the isoenzyme interacts directly with one of the cyclin-cyclin-dependent kinase/inhibitor complexes that regulates entry and completion of S phase. Inappropriate S phase entry has been associated with apoptosis of vascular smooth muscle cells treated with basic fibroblast growth factor antisense oligonucleotides (52), but such apopto-
sis was not found in PKC δ EC. Manipulation of PKC activity has not been previously associated with this S phase defect and only rarely with any abnormality of cell cycle function. Overexpression of PKCδ, but not PKCα or PKCζ, in Chinese hamster ovary fibroblasts causes an arrest in G2/M phase of the cell cycle, but only after activation with PMA (21); no defects were seen in S phase. In that study, inhibition of cell cycle progression by PKCδ was attributed to an isoenzyme-specific effect; since the PKC enzymatic activity was much higher in the PKCδ-transfected cells than in the other transfectants, however, it is possible that the observed effect merely reflected an increase in overall enzyme activity. In this study, however, the similarities of enzymatic activity make it likely that the defect in cell cycle progression, as well as differences in adhesion and migration, resulted from interaction of PKCδ with isoenzyme-specific substrates. Thus, in addition to its isoenzyme-specific character, this arrest in S phase appears to be somewhat specific for endothelial cells.

Specific effects of individual PKC isoenzymes on proliferation are known to vary according to cell types. For example, overexpression of PKCα in murine fibroblast cells inhibits proliferation and does not lead to cell transformation (53, 54), but in NIH 3T3 and human breast cancer cell lines, increases in PKCα expression altered the cell morphology, enhanced proliferation, and increased tumorigenicity (23, 55). Thus, the tissue-specific effects of individual PKC isoenzymes are likely to be mediated by substrates or effectors with restricted tissue or subcellular expression. Even within an individual cell type, the substrates with which each isoenzyme interacts differ. A full understanding of the mechanism by which these two individual PKC isoenzymes mediate either enhanced adhesion or migration or decrease proliferation of endothelial cells will require identification of their selective downstream targets. Such identification, together with the assignment of selective endothelial functions to individual PKC isoenzymes provided by this study, would provide essential details critical to our understanding of reendothelialization and angiogenesis.

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