Angiogenin Activates Phospholipase C and Elicits a Rapid Incorporation of Fatty Acid into Cholesterol Esters in Vascular Smooth Muscle Cells†

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ABSTRACT: Angiogenin activates the phosphoinositide-specific phospholipase C (PLC) in cultured rat aortic smooth muscle cells to yield a transient (30 s) peak of 1,2-diacylglycerol (DG) and inositol trisphosphate. Within 1 min, the DG level falls below that of the control and remains so for at least 20 min. A transient increase in monoacylglycerol indicates that depletion of DG may be the consequence of hydrolysis by DG lipase. In addition to these changes in second messengers, a rapid increase in incorporation of radiolabeled tracer into cellular cholesterol esters is observed. Stimulated cholesterol ester labeling is inhibited by preincubation with either the DG lipase inhibitor RHC 80267 or the acyl coenzyme A:cholesterol acyltransferase inhibitor Sandoz 58035. Cells prelabeled with [^{3}H]arachidonate show a sustained increase in labeling of cholesterol esters following exposure to angiogenin. In contrast, cells prelabeled with [^{3}H]oleate show only a transient elevation that returns to the basal level by 5 min. This suggests initial cholesterol esterification by oleate followed by arachidonate that is released by stimulation of the PLC/DG lipase pathway.

Angiogenin is a 14-kDa protein first isolated from HT-29 human colon adenocarcinoma cell conditioned medium (Fett et al., 1985) that induces vascularization in the chick chorioallantoic membrane and rabbit cornea (Fett et al., 1985; Denêfle et al., 1987). The primary sequence of angiogenin is 35% homologous to human pancreatic ribonuclease (Strydom et al., 1985), and yet the two proteins exhibit distinctly different ribonucleolytic activities (Shapiro et al., 1986). Angiogenin is, in addition, a potent inhibitor of cell-free protein synthesis (St. Clair et al., 1987). Inhibition arises from a specific cleavage by angiogenin of 18S RNA in the 40S ribosomal subunit (St. Clair et al., 1988). The physiological significance of these in vitro activities is unclear, for it has not been demonstrated that angiogenin enters viable cells nor that in vivo protein translation is affected. Angiogenin also activates endothelial cell phospholipase C, leading to a transient increase in cellular 1,2-diacylglycerol (DG)[1] from hydrolysis of phosphatidylinositol (PI) (Bicknell & Vallee, 1988).

Here, we describe the action of angiogenin on vascular smooth muscle cells, including its activation of the phosphoinositide-specific phospholipase C and its induction of rapid cholesterol esterification. We propose that the fatty acids esterified with cholesterol following exposure to angiogenin may derive from phosphoinositide hydrolysis.

Materials and Methods

Materials

Angiogenin was isolated from media conditioned by baby hamster kidney cells that were altered to express human angiogenin (Kurachi et al., 1988). The concentration of stock angiogenin solutions was determined as described (Shapiro et al., 1986), and they were shown to be free of endotoxins (<1 ng/mL in mg/mL stocks) by the *Limulus* amebocyte assay (Yin et al., 1972) using the Sigma Chemical Co. "E-TOXATE" kit. Working solutions of angiogenin were prepared immediately before use in DMEM. RASM cells were isolated according to the method of Gunther et al. (1982).

Phosphatidyl[2-^{3}H]inositol 4,5-bisphosphate (PIP_2) (4 Ci/mmol); L-α-phosphatidyl[2-^{3}H]myoinositol (PI) (10 Ci/mmol); D-[2-^{3}H]inositol 1,4,5-trisphosphate (IP_3) (3.3 Ci/mol); [2-^{3}H]myoinositol (12.8 Ci/mmol); [5,6,8,9,11,12,14,15-^{3}H]arachidonic acid (3200 Ci/mmol); [1,2-^{3}H]cholesterol (58 Ci/mmol); [9,10-^{3}H]oleic acid (10 Ci/mmol); [1,2,3-^{3}H]-glycerol (200 mCi/mmol); EN^{3}HANCE; and Econofluor were from New England Nuclear, Boston, MA. Hydrofluor was from National Diagnostics, Manville, NJ. DMEM, calf serum, glutamine, fungizone, gentamicin, and trypsin/EDTA were from MA Bioproducts, Walkersville, MD. L-α-1,2-Dioleoylglycerol, phosphatidylinositol, and lysophosphatidylinositol were from Avanti Polar Lipids Inc., Pelham, AL. 1-Monostearoyl-*rac*-glycerol, cholesterol arachidonate, and cholesterol oleate were from Sigma Chemical Co., St. Louis, MO. Silica gel G plates were from Analtech Inc., Newark, DE; LK5D linear-K silica gel plates were from Whatman, Clifton, NJ. AG-1-X8 (formate form) was from Bio-Rad. Sandoz 58035 was a gift of Dr. Louis G. Lange, III, Washington University Medical Center. All other reagents were from Sigma or Fisher Scientific.

Methods

Cell Culture and Labeling. RASM cells were cultured in DMEM supplemented with gentamicin (50 μg/mL), fungizone

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[1] Abbreviations: ACAT, acyl coenzyme A:cholesterol acyltransferase; AA, arachidonic acid; BSA, bovine serum albumin; DMEM, Dulbecco's modified Eagle's medium; EDTA, ethylenediaminetetraacetic acid; LPI, lysophosphatidylinositol; PI, phosphatidylinositol; PIP, phosphatidylinositol phosphate; PIP_2, phosphatidylinositol bisphosphate; IP, inositol phosphate; IP_2, inositol bisphosphate; IP_3, inositol trisphosphate; IP_4, inositol tetrakisphosphate; RASM, rat aortic smooth muscle; PLC, phospholipase C; DG, 1,2-diacylglycerol; RHC 80267, cyclohexanone *O,O'*-[1,6-hexanediylbis(iminocarbonyl)]dioxime; Sandoz 58035, 3-(decyldimethylsilyl)-*N*-[2-(4-methylphenyl)-1-phenylethyl]propanamide.

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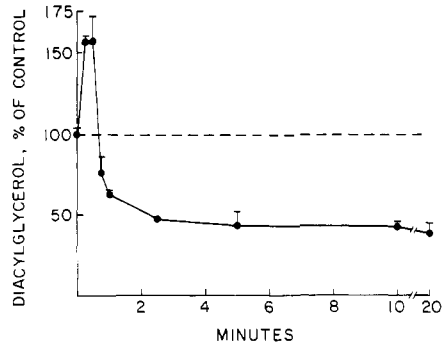

FIGURE 1: Time course of angiogenin-stimulated 1,2-diacylglycerol production in RASM cells. Cells were prelabeled with [^{3}H]arachidonic acid (4 μCi/mL; 48 h), washed, and then exposed to angiogenin (1 ng/mL) or to DMEM alone at 37 °C. Results expressed as percent of controls. Error bars = ±SEM. Control cpm at t_0 = 4500; n = 2.

(0.5 μg/mL), and 10% heat-inactivated calf serum, at 37 °C in a humidified 5% CO_2 atmosphere. Cells (up to passage 20) were trypsinized and plated at a density of 10^5 cells per 35-mm plastic culture dish. Cells were labeled at confluence either with [^{3}H]arachidonate or [^{3}H]oleate (4 μCi per dish) or [^{3}H]glycerol (20 μCi per dish) in DMEM/5% calf serum for 48 h or with [^{3}H]cholesterol (2 μCi per dish) in serum-free DMEM 2 days after confluency for 4 h (to obtain labeling primarily of the plasma membrane). For detection of inositol phosphates, cells were labeled at 90% confluency for 72 h with [^{3}H]myoinositol (10 μCi per dish); LiCl (50 mM) was included in the equilibration buffer to inhibit inositol phosphatases (Inhorn & Majerus, 1987) 20 min before exposure to angiogenin.

All monolayers were incubated in serum-free DMEM for 4 h at 37 °C and then equilibrated in fresh unsupplemented DMEM for an additional 20 min at 37 °C before treatment with angiogenin in DMEM. Reactions were stopped by removal of medium and addition of either chloroform/methanol (2:1) (to extract neutral lipids) or chloroform/methanol/HCl (2:1:0.05) (to extract phospholipids). Extracts were analyzed as described by Griendling et al. (1986). Briefly, neutral lipids were resolved against standards on silica gel G plates and visualized with iodine. Bands were scraped into methanol (500 μL), Econofluor (5 mL) was added, and the samples were quantitated by scintillation counting. Phospholipids were resolved against standards on Whatman LK5D linear-K silica gel plates dipped in 1% potassium oxalate/2 mM EDTA, using a chloroform/methanol/4 M NH_4OH (9:7:2) solvent (Gonzalez-Sastre & Folch-Pi, 1968). Plates were sprayed with EN^{3}HANCE, and phospholipids, visualized by autoradiography, were scraped and counted as for neutral lipids. [^{3}H]Inositol phosphates were resolved on AG-1-X8 resin (formate form) (Downes & Michell, 1981). Siliconized glassware was used throughout. Eluates (2 × 5 mL) were quantitated by scintillation counting following addition of Hydrofluor (10 mL). Where indicated, the cells were preincubated with inhibitor as follows: 50 mM LiCl, 20 min; 5 μg/mL Sandoz 58035, 24 h; 100 μM RHC 80267, 20 min.

Results

Time Course and Dose–Response of Angiogenin-Stimulated Diacylglycerol Formation in Cultured RASM Cells. Angiogenin (1 ng/mL) stimulates up to a 166% increase in DG at 30 s in RASM cells prelabeled with [^{3}H]arachidonate. DG then rapidly falls to 50% of control and remains depleted for up to 20 min (Figure 1). The dose–response at 30 and 60 s (Figure 2) shows that the induction of DG is observed at 30

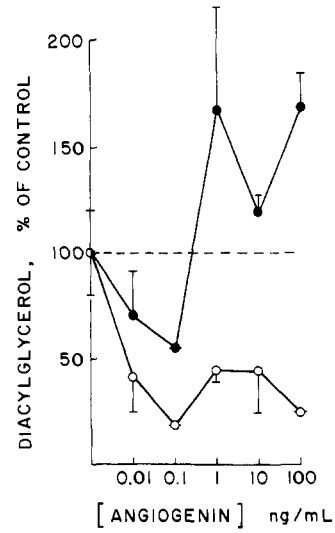

FIGURE 2: Dose–response of angiogenin-stimulated 1,2-diacylglycerol production in RASM cells at 30 (●) and 60 s (○). Cells were prelabeled, and results are expressed as in Figure 1. Error bars = ±SEM. Control cpm at 30 s = 2869, 60 s = 3335; n = 3.

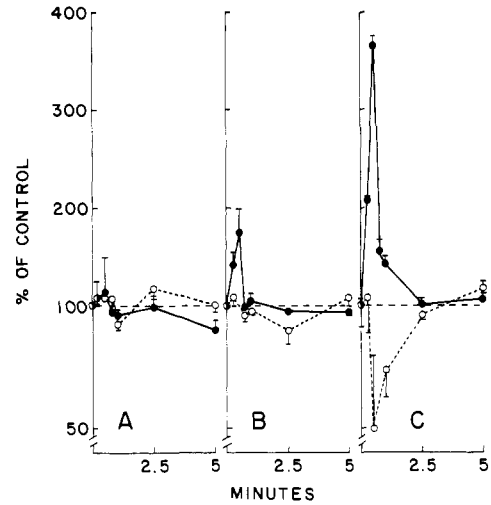

FIGURE 3: Time course of angiogenin-stimulated changes in inositol phosphates (●) and phosphoinositides (○) (in the presence of LiCl) in RASM cells. Cells were prelabeled with [^{3}H]myoinositol (10 μCi/mL; 72 h), washed, preincubated with LiCl (50 mM; 20 min), and then exposed to angiogenin (1 ng/mL) or DMEM alone at 37 °C. Results are expressed as percent of controls. Error bars = ±SEM. Inositol phosphates: (A) IP; (B) IP_2; (C) IP_3. Control cpm at t_0: (A) 27284; (B) 1022; (C) 2222; n = 2. Phosphoinositides: (A) PI; (B) PIP; (C) PIP_2. Control cpm at t_0: (A) 224420; (B) 8921; (C) 10082; n = 2.

s with an angiogenin concentration from 1 to 100 ng/mL. The large standard errors in the induced DG probably reflect the rapid changes in DG level with first a sharp increase followed within 1 min by a decrease to a level below that of controls. The same pattern of changes was observed in several experiments. Intracellular triacylglycerol did not change over 20-min exposure to angiogenin (data not shown).

Time Course and Dose–Response of Angiogenin-Stimulated Inositol Phospholipid Changes in Cultured RASM Cells. The effects of angiogenin (1 ng/mL) on inositol phosphates and phosphoinositides in [^{3}H]myoinositol-labeled RASM cells are depicted in Figures 3 and 4. No statistically significant change in IP levels occurs during a 5-min exposure to angiogenin, but PI levels increase slightly to 115% at 2.5 min (Figure 3A). IP_2 increases transiently to ~175% of controls at 30 s, while PIP levels do not change significantly over 5 min (Figure 3B). The most striking change is in IP_3 which increases dramatically

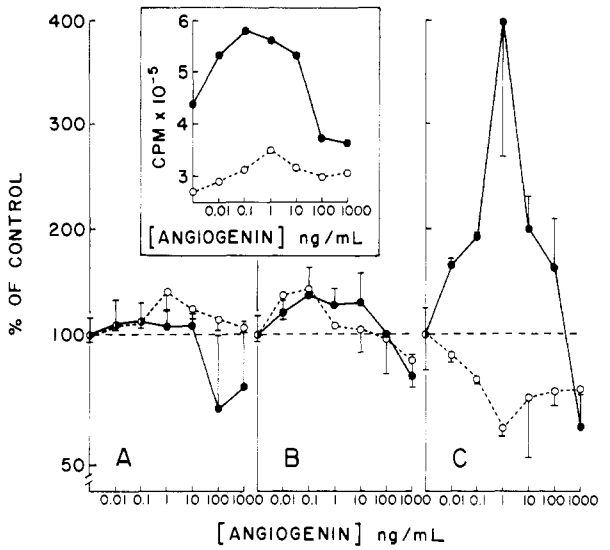

FIGURE 4: Dose–response of angiogenin-stimulated changes in inositol phosphates (●) and phosphoinositides (○) (in the presence of LiCl) in RASM cells prelabeled with [^{3}H]myoinositol as in Figure 3. Results are expressed as percent of controls. Error bars = ±SEM. Inositol phosphates: (A) IP; (B) IP_2; (C) IP_3. Control cpm at 30 s: (A) 43236; (B) 1114; (C) 2978; n = 3. Phosphoinositides: (A) PI; (B) PIP; (C) PIP_2. Control cpm at 30 s: (A) 240180; (B) 16917; (C) 18169; n = 3. Inset = total inositol phosphates (●) and phosphoinositides (○); n = 3.

to 375% of control at 30 s. The absence of statistically significant increases in IP_2 or IP at longer times suggests that under these conditions dephosphorylation of IP_3 is rate-limiting although removal of IP_3 by a previously uncharacterized route cannot be ruled out. There is a correspondingly rapid 50% depletion of PIP_2, but both return to control levels by 5 min (Figure 3C). There were no statistically significant changes in either IP_4 or LPI over 5 min (data not shown).

A maximum increase in total inositol phosphates of ~30% occurs at 30 s at an angiogenin concentration of ~1 ng/mL, but at higher concentrations, total inositol phosphates decrease to ~80% of controls (Figure 4, inset). Total phosphoinositides are also increased maximally by 30% at 1 ng/mL angiogenin, but they remain near basal levels at higher and lower concentrations. Changes induced in the individual IP's and PI's by increasing concentrations of angiogenin are also shown in Figure 4 (panels A–C). Thus, IP and PI levels between 0 and 1000 ng/mL angiogenin (Figure 4A) reflect total inositol phosphate and phosphoinositide levels, as these constitute ~95% of the total cpm. IP_2 increases slightly to ~140% with 0.1–10 ng/mL angiogenin (30 s) but falls at higher concentrations to 84% of controls at 1000 ng/mL. PIP increases to 150% with angiogenin concentrations up to 1 ng/mL, and thereafter it falls to reach 90% of control at 1000 ng/mL (Figure 4B). IP_3 increases to a maximum of 400% at an angiogenin concentration of 1 ng/mL and falls off sharply at higher concentrations. At the same time, PIP_2 decreases to 62% of control up to 1 ng/mL and then returns to 80% of control (Figure 4C). Small changes (up to 25%) were seen in IP_4 (data not shown).

Angiogenin-Stimulated Cholesterol Esterification. In addition to measuring the DG content of extracts from angiogenin-treated RASM cells, we also determined label in the cholesterol ester fraction. Unexpectedly, we found that treatment of the cells with angiogenin (1 ng/mL) increases incorporation of radiolabel into the cholesterol ester fraction to 260% of control within 1 min. In cells labeled with [^{3}H]-arachidonate, this maximal increase occurs at 1 min, somewhat later than for DG, and is preceded by a transient fall in cpm

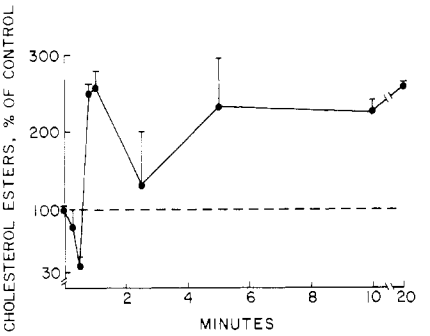

FIGURE 5: Time course of angiogenin-stimulated cholesterol esterification in RASM cells prelabeled with [^{3}H]arachidonic acid as in Figure 1. Results are expressed as percent of controls. Error bars = ±SEM. Angiogenin, 1 ng/mL (—); control (---); n = 2. Control cpm at t_0 = 1300.

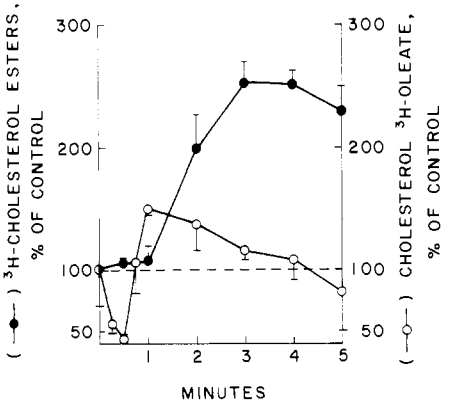

FIGURE 6: Time course of angiogenin-stimulated cholesterol esterification in RASM cells. Cells were prelabeled with either [^{3}H]oleic acid (3.6 μCi/mL for 48 h) (○) or [^{3}H]cholesterol (2 μCi/mL for 4 h) (●), washed, and then exposed to 10 or 1 ng/mL angiogenin, respectively. Results are expressed as a percent of controls. Error bars = ±SEM. Control cpm at t_0: cholesterol [^{3}H]oleate = 2599, n = 2; [^{3}H]cholesterol ester = 186, n = 5. [Of the [^{3}H]cholesterol label added, 0.59% (250% over control) was esterified at 4 min in the presence of angiogenin (1 ng/mL).]

in the cholesterol ester fraction to 30% of control. After peaking at 1 min, the counts in the cholesterol ester fraction drop back to 130% but then rise again to 230% at 5 min and remain elevated at 20 min (Figure 5). In contrast, cells labeled with [^{3}H]oleate, but otherwise treated identically, undergo an initial 50% decrease in label in the cholesterol ester fraction and then an increase to 150% at 1 min and a gradual decrease to 80% of control by 5 min (Figure 6). Cells labeled with [^{3}H]cholesterol for 4 h also show an increase in labeled cholesterol esters in response to angiogenin. In this case, however, there is no initial decrease, and the maximum increase of 250% occurs at 3 min (Figure 6).

The dose dependence of cholesterol esterification was determined at 1 min using RASM cells that were labeled either with [^{3}H]arachidonate (48 h) or with [^{3}H]cholesterol (4 h). In both experiments, incorporation of radiolabeled tracer into cholesterol ester increased with increasing concentrations of angiogenin so that at 100 ng/mL for [^{3}H]arachidonate-labeled cells and at 1000 ng/mL for [^{3}H]cholesterol-labeled cells the cpm in the cholesterol esters are ≃300% of controls (Figure 7). Over the concentration range studied, cells labeled with [^{3}H]arachidonate reach a dose–response plateau whereas cells labeled with [^{3}H]cholesterol do not. Labeling with [^{3}H]-cholesterol for longer times did not alter the profile of esterification.

Angiogenin-Stimulated Monoacylglycerol Formation in RASM Cells. The presence of RHC 80267, a DG lipase

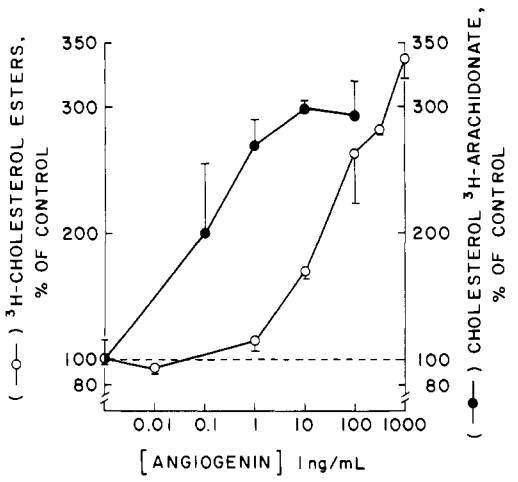

FIGURE 7: Dose–response of angiogenin-stimulated cholesterol esterification (at 1 min) in RASM cells prelabeled with either [^{3}H]-arachidonic acid (●) or [^{3}H]cholesterol (○) as in Figure 6. Results are expressed as percent of controls. Error bars = ±SEM. Control cpm at t_0: (●) 3042; (○) 510; n = 3.

Table I: Effect of DG Lipase and ACAT Inhibitors on Angiogenin-Stimulated Cholesterol Esterification and 1,2-Diacylglycerol Level in RASM Cells[a]

inhibitor	target	CE (%)	DG (%)
none		278	64
RHC 80267	DG lipase	86	362
Sandoz 58035	ACAT	54	90

[a] Cells were prelabeled with [^{3}H]arachidonic acid (4 μCi/mL; 48 h), treated with inhibitors as described under Methods, and then exposed to angiogenin (100 ng/mL) for 1 min at 37 °C (n = 3). Results expressed as percent of buffer or inhibitor control. CE = cholesterol esters.

inhibitor, blocks angiogenin-stimulated cholesterol esterification (Table I). This suggests that the fatty acid that becomes esterified onto cholesterol derives from the breakdown of DG. Increased DG lipase activity in response to angiogenin should be accompanied by an increase in cellular monoacylglycerol. Hence, RASM cells labeled for 48 h with [^{3}H]glycerol were exposed to angiogenin (1 ng/mL) for various times. A transient peak of DG is again observed, followed now by a transient peak of monoacylglycerol (Figure 8), consistent with the sequential action of PLC and DG lipase. The transient nature of the appearance of monoacylglycerol suggests that a monoacylglyceride lipase is also active, which may in turn liberate a second fatty acid that may become esterified onto cholesterol.

Effect of Metabolic Inhibitors on Angiogenin-Stimulated Cholesterol Esterification. RASM cells labeled with [^{3}H]-arachidonic acid for 48 h were incubated with enzyme-specific inhibitors and exposed to angiogenin (100 ng/mL) for 1 min, and DG and cholesterol ester fractions were analyzed. The DG lipase inhibitor RHC 80267 (Sutherland & Amin, 1982) completely blocks angiogenin-stimulated cholesterol esterification (Table I) and allows the DG levels to increase to 362% of the control. Cholesterol esterification is also inhibited (50%) by preincubation of cells with the specific ACAT inhibitor Sandoz 58035 (Ross et al., 1984).

Discussion

Activation of the RASM Cell Inositol-Specific Phospholipase C by Angiogenin. RASM cells respond to low concentrations of angiogenin by generating a rapid and transient increase in cellular DG levels which subsequently fall below control levels (Figure 1). These changes in DG are accompanied by alterations in cellular inositol phosphates and

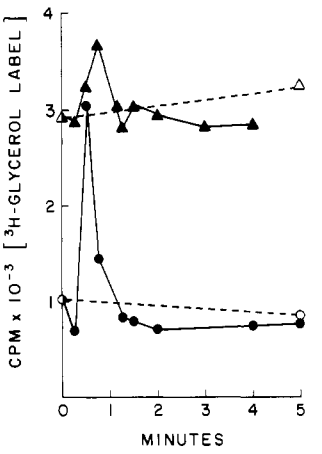

FIGURE 8: Time course of angiogenin-stimulated monoacylglycerol (▲) and 1,2-diacylglycerol (●) production in RASM cells. Cells were prelabeled with [^{3}H]glycerol [20 μCi mL^{-1} (48 h)$^{-1}$], washed, and then exposed for various times to angiogenin (1 ng/mL) at 37 °C. Controls (---); n = 2.

phosphoinositides, most prominently IP_3 and PIP_2[2] (Figure 3).

The results indicate that angiogenin activates a PLC which uses PIP_2 as its major substrate, for only PIP_2 shows a substantial decrease with increasing angiogenin concentrations. This contrasts with the response of endothelial cells to angiogenin where PI is the major substrate for PLC (Bicknell & Vallee, 1988). It may well be that different PLC's are activated by angiogenin in endothelial versus RASM cells. Five types of PLC have been characterized in various tissues [see Rhee et al. (1989)]. Three of the five hydrolyze both PI and PIP_2, but PIP_2 is the preferred substrate at low Ca^{2+} concentrations.

In addition to the decrease in PIP_2, there is a substantial increase in the total phosphoinositide content of RASM cells at 30 s after exposure to angiogenin, indicating de novo PI synthesis (Figure 4, inset). With concentrations of added angiogenin between 0.01 and 10 ng/mL, only PIP_2 appears to be hydrolyzed. Any PI and PIP hydrolysis could be masked by synthetic replenishment; small increases observed in both IP and IP_2 suggest some PI and PIP may be hydrolyzed but this could also be due to the action of a phosphatase on IP_3. Angiogenin-induced (1 ng/mL) hydrolysis of phosphoinositides is supported by a 300% increase in IP_3 at 30 s and a 75% increase in IP_2. At higher angiogenin concentrations, the increase in IP, IP_2, and IP_3 levels is less marked.

In summary, angiogenin at concentrations up to 10 ng/mL activates RASM cell PLC to give a transient increase in the second messengers, DG and IP_3. It has not been determined whether the IP_3 formed is the 1,4,5 isomer which releases calcium from intracellular stores [see Berridge (1987)]. However, we have been unable to detect any internal Ca^{2+} release by monitoring the fluorescence of Fura-2-labeled RASM cells (unpublished observations).

Angiogenin Activation of DG Lipase. The transient nature of the increase in DG induced by angiogenin is at least partially due to the action of DG lipase as indicated by the subsequent increase in monoacylglycerol. A PLC/DG lipase activation has previously been reported in thrombin-stimulated platelets (Prescott & Majerus, 1983). As depicted schematically in

[2] The level of PIP_2 decreased by 50% at 30 s and returned to control levels by 2 min. PIP and PI levels did not decrease with time, but data from the dose response indicate that PI levels increase by 40% over control at 1 ng/mL angiogenin and PIP increases by 50% at 0.1 ng/mL. At higher concentrations, both PI and PIP decrease from their elevated levels, while PIP_2 partially recovers from its depleted state.

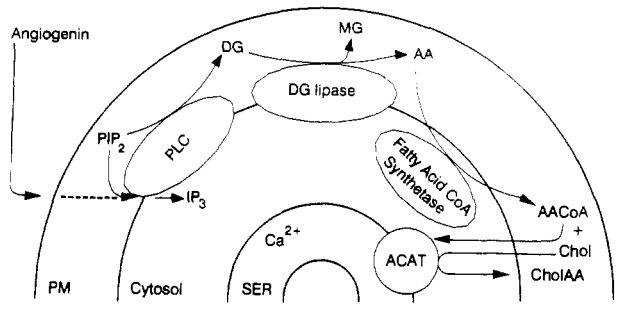

FIGURE 9: Proposed smooth muscle cell response to angiogenin. The mechanism of transduction is not yet characterized, as indicated by dashed arrows. Abbreviations: PM, plasma membrane; SER, smooth endoplasmic reticulum; PIP_2, phosphatidylinositol bisphosphate; PLC, phospholipase C; DG, diacylglycerol; IP_3, inositol trisphosphate; AA, arachidonate; MG, monoacylglycerol; AACoA, arachidonyl coenzyme A; Chol, cholesterol; ACAT, acyl coenzyme A:cholesterol acyltransferase; CholAA, cholesterol arachidonate.

Figure 9, the arachidonic acid released from DG by the action of DG lipase may become esterified with cholesterol by a stepwise conversion to arachidonoyl-CoA (by the action of a fatty acid-CoA synthetase) which then acts as a substrate for ACAT (Lichtenstein & Brecher, 1980). This is supported by the observation that inhibitors of DG lipase and ACAT inhibit angiogenin-induced cholesterol esterification. The cholesterol that becomes esterified in response to low-density lipoprotein (LDL) is known to be oxidase-accessible and therefore thought to be plasma membrane derived (Tabas et al., 1988). Perhaps fatty acid released from membrane lipids would only have access to membrane cholesterol. How the membrane cholesterol and fatty acid are delivered to the microsomal site of ACAT (Balasubramaniam et al., 1978) is unknown. Alternatively, a separate ACAT enzyme may exist in the plasma membrane.

Incorporation of Fatty Acids into Cholesterol Esters. An increase in the incorporation of radiolabeled fatty acid into cholesterol esters is generally taken as evidence for an increase in cell cholesterol ester mass (Brown et al., 1975), however, in the absence of mass quantitation, the possibility that an increase in label arises from a redistribution of fatty acid in the cell cannot be excluded. Regardless, angiogenin clearly stimulates esterification which could result from an activation of ACAT, from an increase in ACAT substrates, or from an inhibition of cholesterol esterase. The mechanism of in vivo ACAT activation is unknown. In vitro, however, increasing concentrations of cholesterol and presumably fatty acid are sufficient to increase ACAT activity [see Chang et al. (1986)]. It is interesting to note that Tabas and Boykow (1987) activated peritoneal macrophage ACAT with LDL by inhibiting protein synthesis with cycloheximide, puromycin, or actinomycin D. Both they and Chang et al. (1986) have proposed that ACAT activity may be suppressed by a short-lived protein inhibitor. Although angiogenin is also an inhibitor of cell-free protein synthesis (St. Clair et al., 1987), there is no evidence to date that it can enter intact cells to disrupt ribosomal function. ACAT activity is also sensitive to free fatty acids [concentrations >50 μM are inhibitory (Goodman et al., 1964)] and 25-hydroxycholesterol [concentrations >100 μM are stimulatory (Erickson et al., 1980)].

The speed (minutes instead of hours) with which angiogenin stimulates cholesterol esterification compared to LDL may reflect the rapid induction of fatty acid substrate for ACAT by angiogenin compared to that delivered by LDL. Alternatively, the increase in cholesterol ester label could be due to inhibition of cholesterol ester hydrolase. The enzyme is activated by the cAMP-dependent protein kinase (Boyd et al., 1983); thus, inactivation could be due either to activation of a protein phosphatase or to a fall in cellular cAMP diminishing the cAMP-dependent protein kinase activity. A decrease in cAMP has been observed in RASM cells in response to angiogenin (Xiao et al., 1989).

Possible Physiological Role(s) of Angiogenin-Stimulated Cholesterol Esterification in RASM Cells. The physiological significance of angiogenin-induced cholesterol esterification remains to be clarified. Among several alternative possibilities, it could serve to compartmentalize excess free cellular arachidonate, thereby preventing its esterification to phospholipids or entry into the eicosanoid synthetic pathway [see review of Irvine (1982)]. Conversely, cholesterol arachidonate could be a source of arachidonate for phospholipid esterification or for entry into the eicosanoid pathway (Beitz et al., 1984). Either mechanism would modulate phospholipid and/or eicosanoid synthesis.

A localized increase in plasma membrane cholesterol esters or an alteration in the fatty acid esterified could induce changes in plasma membrane bilayer fluidity and lateral movements of lipids and proteins, thereby altering rates or types of membrane–protein interactions. This has been proposed by Griendling et al. (1987) for the sustained DG production induced by angiotensin II in RASM cells. Cholesterol bilayer concentrations have been demonstrated to be important in modulating both the activation and deactivation of the hexose transporter in a liposome system (Connolly et al., 1985). Clearly, any of the functions proposed here for cholesterol ester formation induced by angiogenin may modulate the effects of other growth factors on the cell [including mitogenesis; see Heath et al. (1989)]. They may also modulate secretory processes and thus alter the interactions between neighboring cells. In the vasculature, communication of incoming and outgoing signals between and within endothelial and smooth muscle cells may be necessary in the repair and maintenance of tissue (Sporn & Roberts, 1988).

Implications of Angiogenin-Stimulated Cholesterol Esterification in Atherosclerosis. Arterial smooth muscle cells accumulate abnormally high cholesterol ester levels during development of atherosclerosis where lesions also display increased smooth muscle cell proliferation (Small, 1977). At low concentrations, angiogenin stimulates cholesterol esterification and enhances a primary mitogenic stimulus in vascular smooth muscle cells (Heath et al., 1989). The physiological conditions under which vascular smooth muscle cells are exposed to low, subplasma concentrations of angiogenin need to be determined in order to evaluate whether cholesterol esterification is an undesirable secondary effect or an essential part of some mitogenic vessel repair mechanism that becomes uncontrolled when elevated cholesterol and fatty acids are present.

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Registry No. PLC, 9001-86-9; IP_3, 85166-31-0; DG lipase, 83137-80-8; cholesterol, 57-88-5; [^{3}H]arachidonate, 506-32-1; oleate, 112-80-1.

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