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Angiogenin stimulates endothelial cell prostacyclin secretion by activation of phospholipase A_2

(angiogenesis)

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ABSTRACT **Angiogenin stimulates capillary and umbilical vein endothelial cell prostacyclin secretion but not that of prostaglandins of the E series. The response was quantitated by radioimmunoassay and by [^{3}H]arachidonate labeling followed by analysis of the secreted prostaglandins. The stimulated secretion lasts for several minutes and is optimal at 2–4 min. The dose–response (peak at 1–10 ng/ml) is similar to that previously observed for activation of endothelial cell phospholipase C. Stimulated secretion was blocked by pretreatment with the inhibitors of prostacyclin synthesis, indomethacin and tranylcypromine, and also the specific inhibitor of phospholipase A_2, quinacrine, as well as pertussis toxin and the diglyceryl and monoglyceryl lipase inhibitor RHC 80267. Stimulated secretion was also abolished in cells that were either pretreated for 48 hr with phorbol ester to down-regulate protein kinase C or incubated with the protein kinase inhibitor H7. Hydrolysis of phosphatidylinositol by phospholipase A_2 appears to be the source of angiogenin-mobilized arachidonate; angiogenin-induced hydrolysis of phosphatidylcholine was not detected. Activation of phospholipase A_2 occurs in the absence of an angiogenin-induced calcium flux. The results are discussed in terms of mechanisms of agonist-induced intracellular arachidonate mobilization and relevance to angiogenesis.**

Angiogenin is a 14-kDa protein with sequence homology to pancreatic ribonuclease (1) that exhibits low and characteristically different ribonuclease activity (2) and is present in plasma at a relatively high concentration (3). It induces angiogenesis *in vivo* (4) and activates endothelial cell phospholipase C (PLC) and phospholipase A_2 (PLA_2) (5).

Prostacyclin is a prostaglandin secreted primarily by vascular endothelial and smooth muscle cells. It is a potent vasodilator and inhibitor of platelet aggregation and has a relatively short half-life of 2–3 min *in vivo*. Prostacyclin secretion by cultured endothelium is stimulated by numerous agents including thrombin (6, 7), histamine (8), platelet-derived growth factor (9), tumor necrosis factor (10), interleukin 1 (11), high density lipoprotein (12), and bradykinin (13), as well as certain other nonphysiological agents including the calcium ionophore A23187 and trypsin (6).

We report here that angiogenin also stimulates a burst, lasting several minutes, of prostacyclin secretion in cultured bovine adrenal capillary endothelial (BACE) and human umbilical vein endothelial (HUVE) cells. Inhibitors of arachidonate metabolism have been used to delineate the mechanism of angiogenin-stimulated prostacyclin secretion.

MATERIALS AND METHODS

Materials. ^{3}H RIA kits for determination of 6-oxo-$PGF_{1\alpha}$, PGE_1, and PGE_2 (PG, prostaglandin) were from Advanced Magnetics (Cambridge, MA). 6-Oxo-$PGF_{1\alpha}$, PGE_1, PGE_2, indomethacin, tranylcypromine, quinacrine, dexamethasone, pertussis toxin, and phorbol 12-myristate 13-acetate (PMA) were from Sigma. Phosphatidylcholine (PC) and lysophosphatidylcholine (LPC) were from Avanti Polar Lipids. Fura-2 acetoxymethyl ester was from Molecular Probes. H7 was from Seikagaku America (Saint Petersburg, FL). [^{3}H]Phorbol dibutyrate, [^{3}H]choline chloride, and $^{45}CaCl_2$ were from New England Nuclear. RHC 80267, an inhibitor of diacylglycerol (DG) lipase (14), was a gift of S. Prescott (University of Utah School of Medicine). All other materials were as described (5). BACE cells were used only up to passage 15 (when split 1:3) since at higher passage the response to angiogenin was lost.

Methods. Cell culture and DG analysis have been described (5). All cells were incubated in serum-free Dulbecco's modified Eagle's medium (DMEM) for at least 3 hr before use. To determine whether angiogenin induced hydrolysis of PC, BACE cells were labeled with [^{3}H]choline chloride (41.6 μCi per 10^5 cells; 1 Ci = 37 GBq) for 72 hr in 10% fetal bovine serum without endothelial cell growth supplement. Cells were treated with angiogenin, extracted with $CHCl_3$/MeOH/concentrated HCl (1:2:0.05), and the extracts were processed as described (15). PC and LPC were resolved against standards on silica gel plates dipped in borax (10 mM) using a chloroform/methanol/water/ammonia (120:75:8:4) solvent. Analysis of secreted arachidonate metabolites was performed as follows: confluent "cobblestone" monolayers in 35-mm dishes were washed two times with Hanks' balanced salt solution (without bicarbonate and phenol red) and then exposed to agonist in the same medium. Supernatants (2 ml) were collected and spun (1100 × *g*; 15 min), and the top 1.5 ml was removed and either analyzed by RIA or extracted (16) and analyzed by TLC (17). Controls were treated identically. To determine the effect of inhibitors on the cellular response to angiogenin, monolayers of cells were preincubated before exposure to angiogenin with either RHC 80267 (100 μM; 1 hr), indomethacin (20 μM; 1 hr), tranylcypromine (500 μg/ml; 30 min), quinacrine (40 μM; 1 hr), activated pertussis toxin (400 ng/ml; 3 hr), or H7 (100 μM; 30 min) in serum-free DMEM and in the case of dexamethasone (18 hr; 10 μM) was added directly to the medium in which the cells had become confluent. RHC 80267, quinacrine, tranylcypromine, and H7 were present at the indicated concentration during exposure to angiogenin.

Down-regulation of protein kinase C (PKC) in BACE cells, confirmed by [^{3}H]phorbol dibutyrate binding (18), was achieved by incubation with PMA (100 ng/ml) for 48 hr before treatment with angiogenin.

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Abbreviations: BACE, bovine adrenal capillary endothelial; HUVE, human umbilical vein endothelial; PLC, phospholipase C; PLA_1, phospholipase A_1; PLA_2, phospholipase A_2; PKC, protein kinase C; DG, diacylglycerol; PC, phosphatidylcholine; LPC, lysophosphatidylcholine; PG, prostaglandin; PMA, phorbol 12-myristate 13-acetate; G protein, guanine nucleotide-binding regulatory protein.

Agonist-induced Ca^{2+} flux determinations were carried out either by monitoring fluorescence of BACE monolayers with a SPEX-2 fluorimeter following incubation with fura-2/acetoxymethyl ester (5 μM; 30 min) (bradykinin was used as a positive control) or by measurement of calcium efflux after labeling with $^{45}CaCl_2$ (5 μCi per 10^5 cells; 18 hr).

RESULTS

Angiogenin Stimulated Secretion of Arachidonate Metabolites. Low concentrations of angiogenin (1 ng/ml) stimulate a release of ^{3}H-labeled material from BACE and HUVE cells that had been prelabeled with [^{3}H]arachidonate (Fig. 1). Stimulated secretion reached a maximum at 3–5 min and fell to that of control levels by 10 min. No significant increase above controls was seen at longer times up to 24 hr. After incubation of HUVE cells with either pertussis toxin or indomethacin, angiogenin (100 ng/ml; 5 min) failed to stimulate increased secretion of ^{3}H-labeled material [angiogenin-stimulated secretion (above basal secretion): control, +7660 ± 1250; pertussis toxin treated, +1560 ± 1750; indomethacin treated, +1225 ± 1390]. The indomethacin concentration (20 μM) was sufficient to inhibit the arachidonate cyclooxygenase but not PLA_2 (19). In contrast, pertussis toxin did not inhibit the induction of DG (at 2.5 min) by angiogenin (5) (induced DG: angiogenin +4520 ± 1163; pertussis toxin treated + angiogenin, +4404 ± 1664; basal DG, 5247 ± 733).

TLC analysis showed that the major secreted metabolites were prostaglandins of the E series and 6-oxo-$PGF_{1\alpha}$, in accord with a previous report (20). Angiogenin stimulated an increase in the 6-oxo-$PGF_{1\alpha}$ fraction (Fig. 2*A*)—i.e., stimulated prostacyclin secretion, but not that of the E series prostaglandins or of arachidonate. An increase in the 6-oxo-$PGF_{1\alpha}$ fraction was also observed after treatment of HUVE cells with angiogenin (Fig. 2*B*).

Quantitation of Secreted Prostaglandins by RIA. RIA confirmed that angiogenin indeed stimulates prostacyclin secretion by BACE cells but fails to effect that of either PGE_1 or PGE_2. Fig. 3 shows both the dose–response (at 2.5 min) (*A*) and the time course in response to angiogenin (1 ng/ml) (*B*) of angiogenin-stimulated prostacyclin secretion by BACE cells. The time course for prostacyclin secretion as deter-

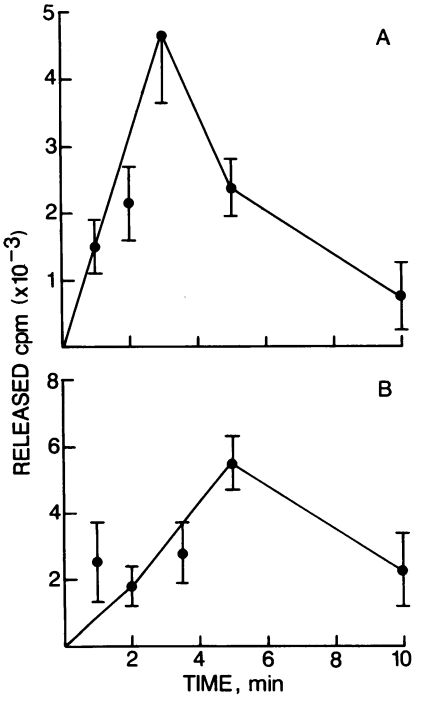

FIG. 1. Time course of angiogenin-stimulated secretion of tritiated metabolites after labeling with [^{3}H]arachidonate (8 μCi/ml; 48 hr) by BACE (*A*) and HUVE (*B*) cells. Conditions: angiogenin, 1 ng/ml (*A*) or 100 ng/ml (*B*) at 37°C. Each point is corrected for basal secretion and represents the mean ± SEM (n = 3).

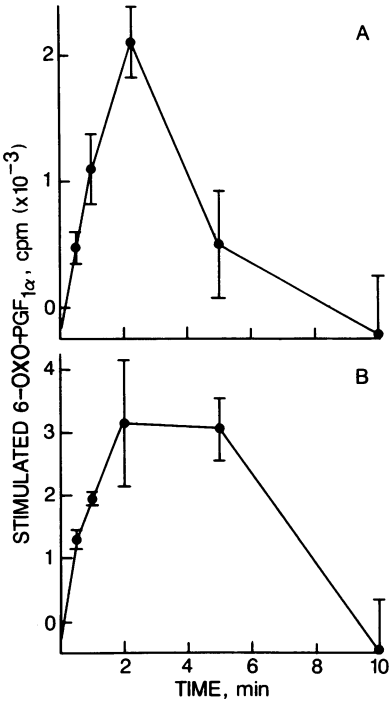

FIG. 2. Time course of angiogenin-stimulated prostacyclin secretion from BACE (*A*) and HUVE (*B*) cells. Cells were labeled with [^{3}H]arachidonic acid (4.15 μCi/ml; 48 hr), washed, and then exposed to angiogenin at 1 ng/ml (*A*) or 100 ng/ml (*B*). Prostacyclin was quantitated after TLC as its stable breakdown product 6-oxo-$PGF_{1\alpha}$. Each point represents the mean ± SEM (n = 3).

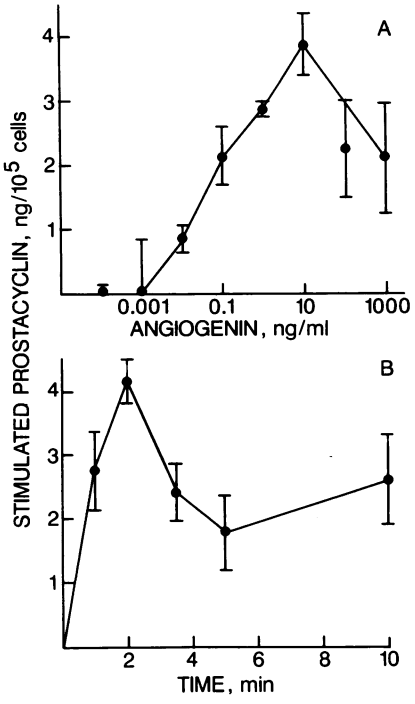

FIG. 3. Dose–response at 37°C and 2.5 min (*A*) and time course at 37°C of angiogenin (1 ng/ml)-stimulated prostacyclin secretion by BACE cells (*B*). Quantitation was by RIA. Basal secretion at 2.5 min was 2 ng per 10^5 cells. Each point represents the mean ± SEM (n = 3).

mined by RIA is comparable to that by labeling with [^{3}H]arachidonate followed by TLC analysis. In contrast, RIA showed that angiogenin did not stimulate release of prostacyclin from the calf pulmonary aortic endothelial cell line despite activation of PLC (5).

Effect of Repeated Exposure to Angiogenin on Endothelial Cell Prostacyclin Secretion. BACE cells were treated three times with angiogenin (1 or 10 ng/ml) for 2 min, with 10-min intervals between exposures followed by RIA quantitation of prostacyclin secretion. Table 1 shows that only the first exposure to angiogenin stimulates the release of prostacyclin.

Effect of Inhibitors of Arachidonate Metabolism on Angiogenin-Stimulated Prostacyclin Secretion. Various inhibitors of arachidonate metabolism effect the angiogenin-stimulated prostacyclin secretion. Indomethacin, tranylcypromine (an inhibitor of prostacyclin synthesis) (6, 21), and RHC 80267 (a DG lipase inhibitor) (14), and the specific PLA_2 inhibitor quinacrine completely abolished the angiogenin-stimulated prostacyclin release (Table 2; data not shown). In contrast, dexamethasone reduced basal secretion but angiogenin-stimulated secretion remained statistically significant. RIA confirmed that activated pertussis toxin blocked stimulated secretion.

Effect of Down-Regulation of PKC by Pretreatment with PMA or Inhibition with H7 on Angiogenin-Stimulated Prostacyclin Secretion. Incubation of BACE cells with PMA (100 ng/ml; 48 hr) abolished the specific binding of [^{3}H]phorbol dibutyrate (specific binding: untreated cells, 1953 cpm; PMA-treated cells, 96 cpm), and we conclude that this treatment is effective in down-regulation of PKC in this cell line. Angiogenin-stimulated prostacyclin secretion does not occur in the phorbol-treated cells (Table 3). Similarly, treatment with the protein kinase inhibitor H7 (22) also abolished angiogenin-stimulated prostacyclin secretion (Table 3).

Source of Angiogenin-Mobilized Arachidonate; Quantitation of PC and LPC Following Exposure to Angiogenin. BACE cells were labeled with [^{3}H]choline and PC and LPC were quantitated after exposure to angiogenin (1 ng/ml; 30-s to 10-min time points). No significant change in either PC or LPC was detected. Thus, phosphatidylinositol appears to be the source of angiogenin-mobilized arachidonate (5).

DISCUSSION

Hydrolysis of phosphatidylinositol by PLA_2 releases free arachidonate from the cellular inositol phospholipid store. The other product of hydrolysis is lysophosphatidylinositol, which has been shown to increase in endothelial cells in response to angiogenin (5). This prompted us to investigate whether angiogenin stimulates arachidonate mobilization in the endothelial cell. BACE and HUVE cells labeled with [^{3}H]arachidonate secrete tritiated metabolites in response to angiogenin (Fig. 1).

Low concentrations of indomethacin (20 μM) inhibit the cyclooxygenase activity of prostaglandin H synthase. At this concentration, indomethacin completely blocks the angiogenin-stimulated secretion of arachidonate metabolites, indicating that the substances secreted exclusively encompass products of the synthase. As reported (20), TLC analysis reveals that PGE_1, PGE_2, and prostacyclin (hydrolyzed to 6-oxo-$PGF_{1\alpha}$ by the acidic extraction medium) are the major arachidonate metabolites secreted by endothelial cells. Comparison of angiogenin-treated samples with controls shows a marked increase in that of 6-oxo-$PGF_{1\alpha}$ (Fig. 2) but not in PGE_1 or PGE_2. RIA confirms these results. There is a short burst of angiogenin-stimulated prostacyclin secretion (Fig. 3) and the dose–response is similar to that previously found characteristic for activation of endothelial cell PLC (5). Stimulated release of prostacyclin is completely blocked by preincubation with either indomethacin or the prostacyclin synthesis inhibitor tranylcypromine.

Agents known to stimulate release of prostacyclin by endothelial cells fall into two categories (Table 4): (*i*) those that stimulate a burst of prostacyclin secretion (lasting <20 min) by acting as mobilizers of arachidonate from intracellular stores (e.g., the inositol phospholipids), and (*ii*) those that promote an increase in the basal level of prostacyclin secretion such that clear differences in the amount of prostacyclin secreted become apparent only several hours after exposure to the agonist.

Cellular synthesis of icosanoids is limited by the availability of their precursor, free arachidonate, which in the case of "short-burst" agonists must be liberated from esterified lipid stores. Four major routes for intracellular arachidonate mobilization have been proposed (see ref. 25 and references therein): (*i*) direct deacylation of phospholipids by PLA_2; (*ii*) deacylation by PLA_1 followed by a lysophospholipase; (*iii*) formation of DG by the action of inositol phospholipid-specific PLC followed by DG lipase; and (*iv*) formation of DG by PLC, phosphorylation by DG kinase to phosphatidate, and then deacylation by a phosphatidate-specific PLA_2. We have previously shown that angiogenin promotes an increase in the lysophosphatidylinositol level of BACE cells consistent with activation of PLA_2 (5). The mechanism of agonist

Table 1. Effect of repeated exposure to angiogenin over a short time period on BACE cell prostacyclin secretion

Angiogenin	Exposure	Prostacyclin, ng per 10^5 cells*
1 ng/ml	1	+4.2 ± 1.0
	2	−2.0 ± 1.0
	3	−1.0 ± 1.5
10 ng/ml	1	+4.2 ± 2.2
	2	0 ± 0.5
	3	0 ± 0.8

Prostacyclin was quantitated by RIA of the breakdown product 6-oxo-$PGF_{1\alpha}$. Cells were treated with angiogenin for 2 min at 37°C, with 10-min intervals between treatments.
*Corrected for basal secretion. Mean ± SEM (n = 3).

Table 2. Effect of metabolic inhibitors on the basal and angiogenin-stimulated prostacyclin secretion by BACE cells

Inhibitor	Prostacyclin, ng per 10^5 cells: Control	Angiogenin treated
None	4.1 ± 0.6	8.9 ± 1.9
RHC 80267 (100 μM; 1 hr)	0.9 ± 0.2	0.8 ± 0.2
Dexamethasone (10 μM; 18 hr)	1.85 ± 0.15	2.9 ± 0.2
Quinacrine (40 μM; 1 hr)	0.25 ± 0.1	0.0 ± 0.3
Pertussis toxin (400 ng/ml; 3 hr)	2.25 ± 0.3	1.6 ± 0.2

Prostacyclin was quantitated by RIA of the breakdown product 6-oxo-$PGF_{1\alpha}$. Conditions for incubations: angiogenin (1 ng/ml), 2 min, 37°C. Mean ± SEM (n = 3).

Table 3. Effect of down-regulation of PKC by treatment with PMA or inhibition of PKC by H7 on angiogenin-stimulated prostacyclin secretion from BACE cells

Treatment	Prostacyclin, ng per 10^5 cells: Control	Angiogenin treated
None	1.44 ± 0.4	4.44 ± 0.6
PMA (100 ng/ml; 48 hr)	3.6 ± 0.8	2.4 ± 0.6
H7 (100 μM; 30 min)	1.44 ± 1.2	1.46 ± 1.3

Prostacyclin was determined by RIA of the breakdown product 6-oxo-$PGF_{1\alpha}$ following a 2-min exposure to angiogenin (1 ng/ml) at 37°C. Mean ± SEM (n = 4).

Table 4. Maximal stimulation of endothelial cell prostacyclin secretion by various agonists

Agonist	-fold stimulation	Time, min	Source or ref.
Rapid release agonists			
Angiogenin	3*	3	This paper
Thrombin	3†	5	23
Bradykinin	10‡	3	13
Histamine	15†	20	24
Slow release agonists			
Tumor necrosis factor	2.7†		10
Interleukin 1	32†		11
Platelet-derived growth factor	74‡ 15*		9
High density lipoprotein	5§		12

The -fold stimulation values for slow release agonists were all determined at 24 hr.
*BACE.
†HUVE.
‡Bovine aortic endothelium.
§Porcine aortic endothelium.

activation of PLA_2 is as yet unclear. Proposals include (*i*) a calcium flux (endothelial cells) (23, 26, 27), (*ii*) by DG-dependent PKC of Madin–Darby canine kidney cells (28), (*iii*) directly by DG—i.e., independent of PKC of 3T3 fibroblasts (29), (*iv*) by guanine nucleotide-binding regulatory protein (G protein) transduction of platelets (30), (*v*) by DG-dependent PKC phosphorylation of the intracellular PLA_2 inhibitor lipocortin (negating its inhibitory action) in neutrophils (31), and (*vi*) by phosphatidate of platelets (32).

The absence of a detectable calcium mobilization in BACE cells after exposure to angiogenin either by fura-2 labeling and fluorescence measurements or by determination of $^{45}Ca^{2+}$ efflux (R.B., unpublished observations) would seem to exclude this pathway for angiogenin activation of PLA_2.* This is in marked contrast with the effect of both bradykinin and thrombin on endothelial cells, where a calcium flux is postulated to be the critical event leading to activation of PLA_2 and release of arachidonate for prostacyclin synthesis (23, 26, 27, 33). We are not aware of a precedent for agonist activation of endothelial cell PLA_2 that does not also induce a calcium flux. In contrast, collagen has been shown to activate platelet PLA_2, releasing arachidonate from phosphatidylcholine, at or near basal levels of calcium (25, 34).

Further studies with angiogenin have shown that stimulation of prostacyclin secretion from BACE cells is blocked by pretreatment with either pertussis toxin, quinacrine, a specific inhibitor of PLA_2 (32), RHC 80267, H7, or PMA to down-regulate PKC. Pertussis toxin has no effect on the induction of DG by angiogenin. These data tentatively suggest that a putative angiogenin receptor is coupled by a pertussis-sensitive G protein to PLA_2, and that PLA_2 is the enzyme that mobilizes arachidonate. G proteins that couple to PLA_2 are generally found to be pertussis sensitive (30, 35, 36), although at least one exception has been documented (37).

The Mechanism of Angiogenin-Stimulated Arachidonate Mobilization. Angiogenin-stimulated arachidonate mobilization is independent of an agonist-induced calcium flux but requires direct transduction of the message via a pertussis-sensitive G protein to PLA_2. It may also be dependent on activation of PKC. Our earlier studies have shown that in BACE cells DG is the major second messenger following exposure to angiogenin; inositol trisphosphate formation is small, an observation consistent with the absence of a calcium flux (5). Lipocortins are 32- to 39-kDa proteins inducible by glucocorticoids and phosphorylated both *in vitro* and *in vivo* by the epidermal growth factor-stimulated protein kinase and by PKC (see ref. 38 and references therein). Lipocortins inhibit PLA_2, and it has been demonstrated (tentatively) that phosphorylation leads to the loss of inhibitory activity (31, 39). It is possible that angiogenin-induced DG in the endothelial cell activates PKC, which phosphorylates a lipocortin and renders it inactive as an inhibitor of PLA_2.† This is consistent with the observation that dexamethasone, an inducer of endothelial cell lipocortins (41), reduces basal prostacyclin secretion but does not block the stimulation by angiogenin. Nevertheless, recent work has shown that *in vitro* PLA_2 inhibition by lipocortins is indirect, involving sequestration of the phospholipid substrate by the protein rather than a direct interaction with PLA_2 (42). *In vivo* inhibition of PLA_2 by a substrate depletion model may be possible if the enzyme/substrate/inhibitor relationship were highly organized in the plasma membrane (42, 43). The physiological significance of lipocortin inhibition of PLA_2 remains to be clarified.

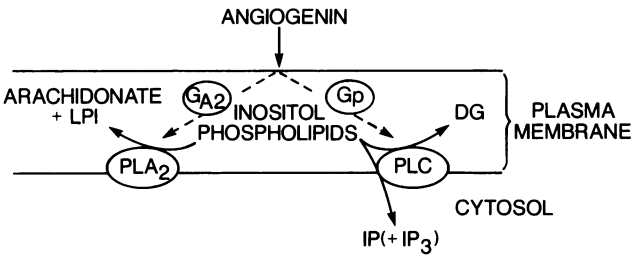

FIG. 4. Mobilization of arachidonate in capillary endothelial cells by angiogenin. Gp, G protein that couples to PLC; G_{A2}, G protein that couples to PLA_2. IP, inositol monophosphate; IP_3, inositol trisphosphate.

Although phorbol esters and the cell-permeant DG analog 1-oleoyl-2-acetylglycerol by themselves do not stimulate PGE_2 release from fibroblasts, they amplify bradykinin-stimulated secretion of this prostaglandin (29) and it has been proposed that DG may activate PLA_2 independently of PKC. Previously, Kramer *et al.* (44) have shown that both 1,2-dioleoylglycerol and 1-stearoyl-2-arachidonylglycerol at a concentration of 1 μM stimulated the calcium-activated platelet PLA_2 >4-fold *in vitro*. Activation of BACE cell PLA_2 by angiogenin-induced DG is an attractive hypothesis; however, as noted above, the loss of response to angiogenin following down-regulation of PKC by prolonged incubation with PMA or inhibition by H7 argues for a role for PKC in angiogenin-stimulated prostacyclin secretion by BACE cells.‡ Fig. 4 summarizes the endothelial cell response to angiogenin leading to mobilization of arachidonic acid.

Only the first exposure to angiogenin (1 or 10 ng/ml) stimulates release of prostacyclin (Table 1), suggesting a specific interaction between angiogenin and a limited number of "receptor" sites on the cell. Once occupied, they must remain so for at least 10 min.

Angiogenin maximally stimulates endothelial cell prostacyclin secretion at concentrations many times less than that present in plasma. If the angiogenin in plasma is active, it

*An angiogenin-induced calcium flux may be either too small, too fast, or possibly spatially localized, such that it has, as yet, escaped detection.

†Type II PKC is activated by DG at basal calcium concentrations (40).

‡H7 is not specific for PKC, inhibiting equally effectively the cAMP- and cGMP-dependent protein kinases (22). However, preliminary experiments have shown no induction of cellular cAMP or cGMP in response to angiogenin (R.B. and Y. Xiao, unpublished observations), and the loss of angiogenin-inducible prostacyclin probably results from H7 inhibition of PKC. Further work is needed to confirm the involvement (or the converse) of PKC in the cellular response.

seems likely that receptor expression is rate limiting. Angiogenin may have a role in the *in vivo* homeostatic secretion of prostacyclin by the endothelium, which is postulated to protect the vessel wall from deposition of platelet aggregates (see ref. 45).

Relevance of Angiogenin-Stimulated Endothelial Cell Prostacyclin Secretion to the *in Vivo* Angiogenic Activity. Two earlier studies have documented the angiogenic activity of prostaglandins of the E series. PGE_2 is angiogenic on the chorioallantoic membrane of the chicken, but not PGA_2, PGF_2, or thromboxane B_2 (46). In the rabbit cornea, PGE_1 and PGE_2 are strongly and slightly angiogenic, respectively, while prostacyclin and $PGF_{2\alpha}$ are completely inactive (47). Furthermore, indomethacin blocks the angiogenic activity of sarcoma-producing fibroblasts in the rabbit cornea, leading to the suggestion that products of prostaglandin synthetase play a role in tumor vascularization. The angiogenic activity of adipocytes and adipose tissue is also prostaglandin dependent (48, 49).

Angiogenin does not stimulate secretion of the angiogenic prostaglandins (PGE_1 and PGE_2) from endothelial cells, even though unstimulated endothelial cells continually secrete these prostaglandins. In contrast, prostacyclin secretion is stimulated by angiogenin. This suggests intracellular organization whereby angiogenin-mobilized arachidonate is selectively directed into prostacyclin synthesis but not into that of PGE_2. The role, if any, of the angiogenin-stimulated prostacyclin secretion in angiogenesis requires further clarification. In this regard, the reported lack of vascularization following corneal implant of prostacyclin (47) must be viewed in light of the fact that prostacyclin is known to be unstable and that the corneal assay requires the substance under investigation to diffuse through the corneal tissue to the vascular limbus before it can elicit a response. Angiogenin-stimulated bursts of prostacyclin secretion in the intimate microenvironment of the capillary vasculature during tissue repair may indeed play a role in angiogenesis. Nevertheless, it is clear that stimulation of prostacyclin secretion alone is unlikely to induce angiogenesis. Of the numerous factors known to stimulate prostacyclin secretion, only tumor necrosis factor has been reported to also be angiogenic (50).

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