Arachidonic Acid, a Growth Signal in Murine P815 Mastocytoma Cells

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ABSTRACT

Evidence is presented that inducing P815 murine mastocytoma cells to grow with serum activates a Ca2+-stimulated phospholipase A2 and the rapid release of arachidonic acid by the cells. Slower growth was also maintained by arachidonic acid or its immediate precursors or by diacylglycerols when bovine serum albumin replaced the serum. Together, arachidonic acid and 1-oleoyl-2-acetylgluceral stimulated growth at the same rate as 10% serum consistent with a role for both arachidonic acid and protein kinase C in the response to serum.

Arresting cell growth with N6,O2-dibutylryladenosine 3',5'-cyclic phosphate and theophylline inhibited the release of arachidonic acid in response to serum, suggesting that cyclic AMP prevents phospholipase activation as one of its pleiotropic effects on growth.

Attempts to demonstrate metabolism of [3H]arachidonic acid to eicosanoids in serum-treated P815 cells by high-performance liquid chromatography or thin layer chromatography were unsuccessful, with the major products being phospholipids and triacylglycerol.

Incubating digitonin-permeabilized P815 cells with [y-32P]ATP and arachidonic acid rapidly increased the phosphorylation of some proteins in the cells, especially the Mr 135,000 and Mr 44,000 proteins which were considerably more phosphorylated than the rest. Phosphorylation of these proteins was not prevented by several inhibitors of protein kinase C, nor was it increased by diacylglycerols or phorbol ester, suggesting that arachidonic acid activates a growth-related protein kinase other than protein kinase C in P815 cells. The possibility that some polyunsaturated fatty acids may promote tumor cell growth by stimulating protein phosphorylation is considered.

INTRODUCTION

Murine P815 mastocytoma cells require serum to grow in culture and they can be induced to arrest in G1 phase and develop morphological characteristics of more mature mast cells by cAMP3 analogues or reagents that increase intracellular cAMP (1–3). Growth arrest is considered.

Over, treating the cells with DBcAMP to arrest growth and induce differentiation inhibited arachidonic acid release. In addition, arachidonic acid alone induced the cells to grow in serum-free medium and it appeared to activate a protein kinase other than protein kinase C in permeabilized cells which could be involved in controlling P815 cell growth.

MATERIALS AND METHODS

Chemicals and Radiochemicals. The following radiochemicals were purchased from NEN: [2-3H]myo-inositol, 12.3 Ci/mmol; [5,6,8,9,11,12,14,15-3H]-arachidonic acid, 180–240 Ci/mmol; [3H]choline, 87 Ci/mmol; 6-keto-5,8,11,12,14,15-3H]prostaglandin Flα, 120–180 Ci/mmol; [y-32P]ATP, 3000 Ci/mmol.

Fraction V BSA was from Park Scientific. BSA alone from some other sources supported growth of the cells. Arachidonic acid was from Nu-chek Prep, Elysian, Leukotrienes and HETE standards were from Cayman Chemical Co. Other biochemicals were supplied by Sigma Chemical Co.

Cell Growth. Cells were grown in RPMI 1640 with 10% neonatal calf serum and growth was arrested with 0.1 mM DBcAMP + 1 mM theophylline (2). Effects of fatty acids or diacylglycerols on growth were measured in 2 ml cultures initiated at 1 x 10⁶ cells/ml. The different compounds dissolved in methanol or dimethyl sulfoxide were added to cultures at or below 0.1% v/v. At these concentrations the solvents alone did not affect growth.

BSA (6 mg/ml) which was added to medium lacking serum did not cause cell growth. Cell growth and viability were determined with a Neubauer hemocytometer before or after staining cells with trypan blue.

Analysis of Serum Effects on Inositol Phospholipids. Log-phase P815 cells (2.5 x 10⁵ cells/ml) were washed with Ca2+/Mg2+-free phosphate-buffered saline and resuspended at 1 x 10⁶ cells/ml in serum-free, isoinol-free medium. [3H]Myo-inositol (1 μCi/ml) was added and the cells were grown for 8 h. Cells recovered by centrifugation were resuspended at 1 x 10⁶ cells/ml in serum-free, isoinol-free medium containing 10 mM LiCl and incubated at 37°C. After 3 min 1 ml aliquots of cells were added to test tubes containing serum (final 10%). Assays were terminated by adding 120 μl of 100% (v/v) trichloroacetic acid to individual tubes after 0, 10, 30, 90, and 270 s and the phosphoinositides and inositol phosphates were extracted and separated on 4-cm AG1-X10 anion exchange columns exactly as described by Creba et al. (8) and Berridge (9), respectively.

Serum-stimulated Arachidonic Acid Release. To determine whether serum-stimulated arachidonic acid release from P815 cells 1 x 10⁶ log-phase cells washed twice with 10 ml RPMI minus serum were resuspended in 17 ml medium minus serum containing 20 mM Na⁺ Hepes buffer (pH 7.4) and 4 μCi [3H]arachidonic acid and grown for 4 h at 37°C. After centrifugation the cell pellets were washed twice with 5 ml medium minus serum containing Hepes buffer and 1 mg/ml BSA and then resuspended in 22 ml Hepes-buffered medium minus serum. Two 0.5 ml aliquots were immediately removed and centrifuged for 20 s in a microfuge, and the radioactivity in 0.2 ml aliquots of the supernatants was measured in Triton X100-toluene-based scintillant. The remaining cell suspension was divided into 4 x 0.5 ml aliquots to which were added 6 mg/ml BSA, 10% serum, or 10% serum with added drugs. Aliquots (0.5 ml) were removed at intervals up to 3 h and centrifuged, and the radioactivity in the supernatants was measured as described above.

To show that cAMP inhibited arachidonic acid release in response to serum, cells were grown with 10% serum and 0.1 mM DBcAMP + 1 μCi theophylline for 14 h to arrest growth, washed, and then grown for 4 h in serum-free medium with 4 μCi [3H]arachidonic acid and DBcAMP + theophylline to label phospholipids and at the same time deplete serum factors. After washing the cells to remove free [3H]arachidonic acid they were resuspended in serum-free medium with DBcAMP + theophylline and divided, and release of [3H]-arachidonic acid was measured after adding 6 mg/ml BSA or 10% serum.

Received 1/25/93; accepted 5/11/93.

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1 This research was supported by a grant from the Cancer Society of New Zealand.

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3 The abbreviations used are: cAMP, cyclic AMP; PI, phosphatidylinositol; HPLC, high-performance liquid chromatography; BSA, bovine serum albumin; DBcAMP, N6, O2-dibutylry adenosine 3',5'-cyclic phosphate and theophylline.
In separate experiments the \(^3\)H material released into the medium after serum addition to serum-deprived \(^3\)H-arachidonic acid-prelabeled cells was recovered and examined by reverse-phase HPLC. Samples were obtained by three methods: (a) SEP-PAK C18 cartridge (10); (b) 80% methanol (11); or (c) ethyl acetate extraction (12). Extracts were fractionated on a Brownlee Spheri 4RP-18 column essentially using the method of Henke et al. (11) for fractionating prostaglandins, leukotrienes, and HETE but with a gradient from 65 to 100% methanol from 55 to 70 min. Authentic eicosanoid standards 5(S)HETE, 8(S)HETE, 11(S)HETE, 12(S)HETE, 15(S)HETE, leukotrienes C4, D4, E4, N-acetyl E4, F4, and 6-ketoprostaglandin F1α were also added to the culture medium, extracted, and fractionated to confirm that the procedures used successfully recovered eicosanoids.

**\(^{3}\)H-Arachidonic Acid Incorporation into Cell Lipids.** To identify products of arachidonic acid metabolism 2–3 × 10\(^5\) log phase or treated cells were washed with 20 m\(\lambda\) Hepes-buffered serum-deficient medium, resuspended in 15 ml of the same medium, and grown for 4 h. After centrifugation the cells were resuspended in 3.2 ml of the medium and divided into 3 1-ml cultures. These cultures were grown with 2 \(\mu\)Ci \(^{3}\)H-arachidonic acid and 6 mg/ml bovine serum albumin or 10% serum for 10 min before the cells were recovered by centrifugation at 4°C and washed with ice-cold medium containing 1 mg/ml BSA, and the cell pellets were extracted 3 times with 0.5 ml methanol:chloroform:formic acid (1:0.5:0.4; v/v). The combined extracts were evaporated in a vacuum centrifuge and the residues were dissolved in 0.1 ml methanol:chloroform (1:1) with vigorous sonication. Aliquots (70–\(\mu\)l) of the extracts were applied to silica gel SiF thin-layer chromatography plates (Riedel-de Haën) which were developed in diethyl ether:petroleum ether:acetic acid (50:50:1) to separate phospholipids from HETE and di- or triacylglycerols or in methanol:chloroform:formic acid (15:65:5; v/v) to resolve different classes of phospholipids. After exposure to iodine vapor to locate lipid standard markers the chromatograms were cut into 0.5-cm strips to measure the associated radioactivity using a toluene-based scintillant. Some extracts were also hydrolyzed with porcine phospholipase A2 (Sigma) prior to chromatography to confirm that \(^{3}\)H-arachidonic acid was present in phospholipids.

**Arachidonic Acid-activated Protein Phosphorylation.** Protein phosphorylation in response to arachidonic acid was measured by the method of Eru-salimsky et al. (13) using \((\gamma-^{32}\)P\)ATP and digitonin-permeabilized cells. Briefly, 3 × 10\(^5\) log-phase P815 cells were recovered by centrifugation, washed twice with 20 m\(\lambda\) Hepes-buffered medium without serum, resuspended in 20 m\(\lambda\) Hepes-buffered medium containing 6 mg/ml bovine serum albumin, and grown at 37°C for 4 h to reduce effects of serum factors. The cells were then washed twice with KCl medium (13) at 37°C resuspended in the same KCl medium and 0.4-ml aliquots (~4 × 10\(^5\) cells) were distributed into separate 15-ml microfuge tubes containing \((\gamma-^{32}\)P\)ATP, digitonin, arachidonic acid, or other reagents and KCl medium in 0.1-ml volume. The reaction mixtures were incubated at 37°C for the appropriate times and then chilled in ice and the cells were pelleted by centrifugation. After removing the supernatants, the cell pellets were dissolved in 0.1 ml standard polyacrylamide gel protein dissociation buffer and the proteins were fractionated on a 7–15% gradient polyacrylamide gel. The fixed Coomassie blue stained gels were dried and autoradiographed using Agfa Curix x-ray film with intensifying screens.

**RESULTS**

**Serum-Induced Arachidonic Acid Release.** Addition of serum to serum-deprived \(^3\)H-arachidonic acid-prelabeled P815 cells caused a substantial increase in the rate of release of \(^3\)H-labeled material from the cells which was inhibited by the phospholipase A2 inhibitors quinacrine or manoalide, consistent with serum activation of phospholipases C or D (14). Briefly, 3 × 10\(^5\) log-phase P815 cells were recovered by centrifugation, washed twice with 20 m\(\lambda\) Hepes-buffered medium without serum, resuspended in 20 m\(\lambda\) Hepes-buffered medium containing 6 mg/ml bovine serum albumin, and grown at 37°C for 4 h to reduce effects of serum factors. The cells were then washed twice with KCl medium (13) at 37°C resuspended in the same KCl medium and 0.4-ml aliquots (~4 × 10\(^5\) cells) were distributed into separate 15-ml microfuge tubes containing \((\gamma-^{32}\)P\)ATP, digitonin, arachidonic acid, or other reagents and KCl medium in 0.1-ml volume. The reaction mixtures were incubated at 37°C for the appropriate times and then chilled in ice and the cells were pelleted by centrifugation. After removing the supernatants, the cell pellets were dissolved in 0.1 ml standard polyacrylamide gel protein dissociation buffer and the proteins were fractionated on a 7–15% gradient polyacrylamide gel. The fixed Coomassie blue stained gels were dried and autoradiographed using Agfa Curix x-ray film with intensifying screens.

**Fig. 2.** Effects of ethyleneglycol bis(\(\beta\)-aminoethyl ether)\(\cdot\)N\(\cdot\)N\(\cdot\)N\(\cdot\)N\(\cdot\)-tetraacetic acid on release of \(^3\)H-labeled material from P815 cells previously preincubated with \(^3\)H-arachidonic acid. A: O, minus serum; +, plus serum; •¿, plus serum and 5 m\(\lambda\) quinacrine; •¿, plus serum and 10 m\(\lambda\) quinacrine; •¿, plus serum and 20 m\(\lambda\) quinacrine. B: O, minus serum; +, plus serum; •¿, plus serum and 10 m\(\lambda\) manoalide; •¿, plus serum and 20 m\(\lambda\) manoalide. C: D: DBcAMP-arrested cells. O, minus serum; +, plus serum.
eluted in 100% methanol (Fig. 3). The latter peaks were identified as phospholipids by thin-layer chromatography before and after hydrolysis with porcine phospholipase A2. Because high speed centrifugation (27,000 × g; 10 min) of the cell supernatants reduced the radioactivity in the latter peaks by 50% without decreasing the peak of [3H]arachidonic acid we believe the phospholipids originated from membrane vesicles produced by P815 cells (15). In five experiments 17.7% (SD, 1.1) of the radioactivity released from cells stimulated with serum for 40 min was arachidonic acid which corresponded to a 10–12-fold increase compared to cells treated with BSA.

There was no indication from the HPLC that [3H]-eicosanoids were produced in growth medium. Although attempt to induce eicosanoid production by growing cells with [3H]arachidonic acid then treating the cells with the Ca²⁺ ionophore A23187, or after incubating cell extracts with [3H]arachidonic acid as described by Koshihara (16) or McMillan et al. (17), did not produce [3H]-eicosanoids, although radioactivity was incorporated into material that cochromatographed with phospholipids and triacylglycerol on thin-layer chromatography or HPLC.

**Growth with Arachidonic Acid.** To ascertain whether arachidonic acid alone could induce growth of P815 cells in serum-deficient medium containing 6 mg/ml BSA were grown with 0–20 μM arachidonic acid. Cultures without arachidonic acid soon died, whereas those receiving 10 or 20 μM arachidonic acid grew at 50–70% of the rate with 10% serum (Fig. 4, A and B). Growth did not occur without BSA in the medium, suggesting the protein was necessary to carry arachidonic acid to the cells. The arachidonic acid precursors oleic, linoleic, or linolenic acids (10 μM) were just as effective as arachidonic acid in stimulating growth, whereas 10 or 20 μM palmitic or stearic acid did not induce growth in medium containing BSA.

Because polyunsaturated fatty acids can activate protein kinase C (18) cells were also grown with the protein kinase C activators 1-oleoyl-2-acetylglycerol, 1,2-dioleoylglycerol, or 1,2-dioctanoylglycerol and BSA in the absence of serum. At 10 μM concentrations each of the diacylglycerols induced the cells to grow, although at reduced rates compared with 10% serum (Table 1) and increasing the concentration from 5 up to 20 μM 1,2-dioleoylglycerol or 1,2-dioctanoylglycerol produced only a small additional increase in growth (Table 1).
Effects on Inositol Phospholipids. To determine whether serum also activated inositol phospholipid turnover cells were grown for 3 h with \(^{3}H\)myo-inositol in the absence of serum, and the distribution of \(^{3}H\)inositol in phosphatidylinositol mono-, di- and Tris-phosphates and inositol mono-, di-, and Tris phosphates was analyzed before and immediately after serum readdition in the presence or absence of 10 mm LiCl (8, 9). There was no significant change in any of the inositol phospholipids or inositol phosphates over 5 min although effects on phospholipid metabolism have been reported within 15 s after stimulation with other cells and substantial changes after 1-2 min (19) (data not shown).

Protein Phosphorylation. Arachidonic acid has been reported to activate protein kinase C either alone or synergistically with diacylglycerols (18, 20). Therefore we used the procedure of Erusalimsky et al. (13) to examine the effects of arachidonic acid upon protein phosphorylation with \(\gamma\text{-}^{32}\text{P}\)ATP in serum-deprived digitonin-permeabilized P815 cells. Ten \(\mu\text{M}\) arachidonic acid rapidly stimulated the phosphorylation of \(M_{r}\) 135,000 and \(M_{r}\) 44,000 proteins and some other more weakly phosphorylated proteins in the permeabilized cells (Fig. 5). Quantitation of the \(M_{r}\) 135,000 and \(M_{r}\) 44,000 bands in the autoradiographs with a Joyce label microdensitometer showed that they were 2.9 ± 0.2- and 1.9 ± 0.2-fold more phosphorylated respectively after 5 min treatment with arachidonic acid whereas phosphorylation of some other proteins was barely affected which precludes a general stimulation of protein phosphorylation by arachidonic acid. Phosphorylation of the \(M_{r}\) 135,000, and \(M_{r}\) 44,000 proteins was not inhibited by the protein kinase C inhibitors H7 (10 or 50 \(\mu\text{M}\)), staurosporine (0.2 \(\mu\text{M}\)), or sphingosine (10 \(\mu\text{M}\)) or by 5 \(\mu\text{M}\) chelerythrine which is a more potent protein kinase C inhibitor. The protein kinase C activators 1-oleoyl-2-acetylglycerol (10 \(\mu\text{M}\)), 1,2-dioleoylglycerol (10 \(\mu\text{M}\)), 1,2-dioctanoyl-glycerol (10 \(\mu\text{M}\)), or 200 and 400 nm phorbol ester, respectively, failed to stimulate protein phosphorylation in the permeabilized cells, nor did 10 \(\mu\text{M}\) 1-oleoyl-2-acetylglycerol plus 10 \(\mu\text{M}\) arachidonic acid increase protein phosphorylation compared to that observed with 10 \(\mu\text{M}\) arachidonic acid alone. The saturated fatty acids palmitic acid (10 \(\mu\text{M}\)) or stearic acid (10 \(\mu\text{M}\)) also did not increase protein phosphorylation (data not shown). Alkaline hydrolysis of gels containing proteins phosphorylated in response to arachidonic acid followed by autoradiography eliminated the radioactive proteins, confirming that they were not phosphorylated on tyrosine residues (21) (data not shown).

When cells arrested by 14 h growth with 0.1 \(\mu\text{M}\) DBcAMP and 1 \(\mu\text{M}\) theophylline were incubated with 10 \(\mu\text{M}\) arachidonic acid, \(\gamma\text{-}^{32}\text{P}\)ATP, and digitonin, there was a substantial reduction in overall protein phosphorylation and stimulation of protein phosphorylation by arachidonic acid was not observed in contrast to the increased protein phosphorylation in untreated cells (Fig. 6). In toto these results suggested that arachidonic acid activates a protein kinase other than protein kinase C in P815 mastocytoma cells and that arresting growth with the cAMP analogue largely eliminated protein phosphorylation in response to arachidonic acid.

DISCUSSION

The above results showed that serum stimulates arachidonic acid release in P815 cells apparently by activating a \(\text{Ca}^{2+}\)-stimulated quinacrine- or manoleide-sensitive phospholipase tentatively identified as phospholipase A2. Since arachidonic acid or its precursors alone also induced growth, albeit at a slower rate than serum, these observations suggest that arachidonic acid released by serum is involved in growth signal transduction in P815 cells.

Either 1-oleoyl-2-acetylglycerol or 1,2-dioleoylglycerol also increased P815 cell growth in the absence of serum suggesting that protein kinase C activation is also involved in stimulating P815 cell growth. Furthermore, because 10 \(\mu\text{M}\) 1,2-dioctanoylglycerol induced slow growth, hydrolysis of 1-oleoyl-2-acetyl- or 1,2-dioleoylglycerol to release oleic acid was probably not responsible for the growth observed with diacylglycerols. Therefore, these results and the greater effect of arachidonic acid and diacylglycerol together on growth (Table 2) suggest that arachidonic acid released by serum transmits part of the growth signal in P815 cells and that protein kinase C activation is required for a complete response.

P815 cells contain protein kinase C (7); therefore, the failure of 1-oleoyl-2-acetylglycerol to activate the enzyme to stimulate protein phosphorylation in the permeabilized cells may have been due to the low concentration of free calcium (15 \(\text{nm}\)) in the solution used to permeabilize the cells (13) or inactivation of protein kinase C by the permeabilization solution. Further studies will be necessary to answer this question.

Despite a considerable effort, we could not detect any conversion of \(^{3}H\)arachidonic acid into eicosanoids by our P815 cells. Consequently our earlier conjecture based on drug inhibition studies that metabolism of arachidonic acid via lipoxygenase might be involved in P815 cell growth appears to be incorrect (22) and the effects of lipoxygenase inhibitors on growth may be due to the antioxidant or other properties of most lipoxygenase inhibitors (23).

In our previous study we found no effect of indomethacin or ace-tylsalicylate on the growth of P815 cells, and since we could not
4. 14 h DBcAMP arrested cells. No arachidonic acid (Lanes 1 and 4); 10 μM arachidonic acid (Lanes 2 and 4). Cells were incubated for 5 min at 37°C prior to polyacrylamide gel electrophoresis fractionation.

Fig. 6. Effect of growth arrest with DBcAMP on protein phosphorylation in permeabilized cells, in response to arachidonic acid. Lanes 1 and 2, log-phase cells; Lanes 3 and 4, 14 h DBcAMP arrested cells. No arachidonic acid (Lanes 1 and 3); 10 μM arachidonic acid (Lanes 2 and 4). Cells were incubated for 5 min at 37°C prior to polyacrylamide gel electrophoresis fractionation.

detect conversion of [3H]arachidonic acid into prostaglandins by the cells metabolism of arachidonic acid to prostaglandins is unlikely to be involved in the action of arachidonic acid on growth (22). These results are not entirely surprising as P815 cells are reported to completely lack cyclooxygenase activity (24) and they also lack the activator protein, FLAP, required for 5-lipoxygenase to oxidize arachidonic acid (25).

Arachidonic acid can directly or synergistically activate some species of protein kinase C (18, 20, 26), it is an inhibitor of the GTPase activator protein GAP (27, 28), it affects membranes and Ca2+ availability (29) and it activates ATP-sensitive K+ channels (30). In addition, Szamel et al. (31) have suggested that incorporation of polyunsaturated fatty acids into plasma membrane phospholipids activates protein kinase C in lymphocytes; therefore, there are many possible ways in which arachidonic acid might promote cell growth. Recently, simultaneous actions of diacylglycerol and unsaturated fatty acids have been shown to activate platelets and it was suggested that cis-unsaturated fatty acids may take part in cell signaling through the protein kinase C pathway (32). In addition, Khan et al. (33) have reported that arachidonic acid and cis-unsaturated fatty acids induce protein phosphorylation in platelets. Although the proteins phosphorylated in response to arachidonic acid were only a subset of those phosphorylated with phorbol-ester the authors concluded that the effect of arachidonic acid involved activation of protein kinase C. However, our results suggest that the action of arachidonic acid on P815 cell growth may be more direct, possibly through stimulation of a kinase other than protein kinase C that is one of the kinases in the protein kinase cascade recently implicated in the control of mammalian cell growth (34, 35). One candidate is Mr 74,000 murine ras-1 kinase since ligand-stimulated kit receptor has been shown to be coupled to ras-1 kinase in NIH 3T3 cells transfected with a chimeric epidermal growth factor-kit receptor then stimulated to grow with epidermal growth factor (36). Furthermore, activation of the chimeric kit receptor did not cause significant changes in inositol phosphate production. After treating permeabilized P815 cells with arachidonic acid there appeared to be an increase in phosphorylation of a barely discernable protein in the Mr 74,000 region of Fig. 5 (arrow) which might correspond to autophosphorylated ras-1 kinase; however, the protein was not identified.

Basudev et al. (37) have recently reported that arachidonic acid induces phosphorylation of an Mr 18,000 protein in electrically permeabilized rat islets of Langerhans, despite down-regulation of protein kinase C; therefore, protein kinase activation by arachidonic acid may be a more common phenomenon.

Receptor-mediated activation of phospholipase A2 and arachidonic acid release and metabolism are recognized as important mechanisms for second messenger production in animal cells (38) and arachidonic acid or its metabolites are required for mitogenesis in several fibroblast cell lines (39, 40). Phospholipase A2 is also involved in mast cell activation (41) and possibly T-cell activation (42), while in other cells ras has been implicated in phospholipase A2 activation (43) and arachidonic acid has been shown to inhibit the ras GTPase activator protein GAP (44). Therefore, failure to regulate correctly the production or products of phospholipase A2 action might well cause the constant growth of P815 cells.

There is suggestive evidence that dietary n-6 polyunsaturated fatty acid may increase the incidence of some types of tumors (45-47). Our data with P815 cells would be consistent with polyunsaturated fatty acids acting via protein kinases to promote tumor cell growth. Hannigan and Williams (48) have reported interferon-α-induced activation of phospholipase A2 and arachidonic acid production in 3T3 fibroblasts. They also concluded that arachidonic acid was not metabolized by cyclooxygenase or lipoxygenase and speculated that arachidonic acid might participate in cytokine-specific growth responses by activating latent transcription factors. Recently evidence has also been presented that epidermal growth factor activates calcium channels by phospholipase A2/5-lipoxygenase-mediated leukotriene C4 production (49) and that stimulation of proliferation of porcine aortic smooth muscle cells by ATP requires a dual mechanism involving arachidonic acid release and protein kinase C (50). Therefore, arachidonic acid may play a more extensive role in growth than has hitherto been recognized.

Finally, in murine spleen-derived PT18 mast cells, cyclic AMP has been shown to inhibit the lipase-catalyzed cleavage of arachidonic acid from membrane phospholipids in the absence of measurable effects on either histamine release or changes in cytosolic calcium concentrations (51) and in other cells cAMP blocked phosphatidylinositol turnover and cellular activation (52). These observations raise the possibility that growth arrest and differentiation induced by cyclic AMP (1, 53) may result from interference with phospholipase activation and growth signal transduction at the cell membrane in addition to effects on the phosphorylation of transcription factors such as CREB protein.

ACKNOWLEDGMENTS

We thank Dr. D. J. Faulkner, P. J. Scheuer, and A. W. Ford-Hutchinson for supplying compounds used in our research.

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