

REGULATION OF ARACHIDONIC ACID METABOLISM, AROMATASE ACTIVITY AND GROWTH IN HUMAN BREAST CANCER CELLS BY INTERLEUKIN-1 β AND PHORBOL ESTER: DISSOCIATION OF A MEDIATORY ROLE FOR PROSTAGLANDIN E₂ IN THE AUTOCRINE CONTROL OF CELL FUNCTION

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Prostaglandin E₂ (PGE₂) levels are elevated in malignant human breast tissue. However, the cellular mechanisms regulating this arachidonate metabolism and the autocrine influence PGE₂ production may have on breast cancer cell growth and function are unclear. In the present study, we have investigated the effects of 2 putative cyclo-oxygenase inducers, interleukin-1 β (IL-1 β) and the protein kinase C agonist 12-O-tetradecanoyl-phorbol-13-acetate (TPA), on PGE₂ production, growth and aromatase activity in the MDA MB 231 breast cancer cell line. TPA stimulated a dose-dependent increase in PGE₂ production, inhibited cell growth and stimulated aromatase activity. Although IL-1 β alone had no effect on any of these breast cancer cell functions, the cytokine greatly potentiated PGE₂ production in the presence of TPA. Similarly, growth inhibition and aromatase stimulation in response to TPA were both further enhanced by IL-1 β treatment. Indomethacin and dexamethasone both prevented PGE₂ production in response to IL-1 β and TPA but had no effect on the anti-proliferative action of the cytokine and phorbol ester. While indomethacin had no effect on induction of aromatase activity by IL-1 β and TPA, dexamethasone exhibited a temporally biphasic action. Dexamethasone alone stimulated aromatase activity and demonstrated a permissive action on aromatase stimulation by IL-1 β and TPA. However, pre-treatment of cells with dexamethasone prevented subsequent induction of aromatase activity by IL-1 β and TPA. Our study describes a novel synergistic interaction in response to protein kinase C activation and IL-1 β during the regulation of arachidonate metabolism, cell growth and aromatase activity in human breast cancer cells. We conclude that the cyclo-oxygenase pathway does not play a mediatory role during the inhibition of cell growth and the induction of aromatase activity by IL-1 β and TPA.

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Arachidonic acid metabolism via the cyclo-oxygenase pathway is often elevated in malignant tissue, and indeed, the presence of high prostaglandin E₂ (PGE₂) levels in human breast cancer tissue is well documented (Karmali *et al.*, 1983). Furthermore, tissue from malignant breast tumours also exhibits a high capacity for PGE₂ production compared with benign or normal tissue (Watson and Chuah, 1992). Nonetheless, our knowledge remains limited with respect to both the factors controlling PGE₂ synthesis in human breast cancer cells and the consequential actions of this eicosanoid on cancer cell growth and function.

Many previous studies employing human and murine mammary cancer cell lines in both *in vitro* and *in vivo* murine tumour models indicate that arachidonate metabolites, including PGE₂, play a multifunctional role in controlling growth (Fentiman *et al.*, 1984; Fulton, 1984), metastasis (Fulton *et al.*, 1991) and host-immune responses (Fulton and Heppner, 1985). Most of these investigations have employed exogenous eicosanoids or have studied the modulatory effects of various inhibitors of arachidonate metabolism during constitutive eicosanoid synthesis. Two forms of cyclo-oxygenase have now been identified, being the products of 2 separate genes (Hla and Neilson, 1992). Cyclo-oxygenase-1 is constitutively expressed, while cyclo-oxygenase-2 is an inducible form which appears to be more important in regulating prostaglandin production by

extracellular ligands (Crofford *et al.*, 1994). Rather than acting as a primary agonist in isolation, PGE₂ often functions as a mediator or feedback modulator during the action of a wide variety of other autocrine/paracrine factors. Hence, the present study was conducted to investigate the potential for such a role for PGE₂ in breast cancer cells during the action of 2 putative activators of cyclo-oxygenase induction, the cytokine interleukin-1 β (IL-1 β) and the protein kinase C agonist 12-O-tetradecanoyl-phorbol-13-acetate (TPA) (Crofford *et al.*, 1994). Both IL-1 β and TPA have been reported to elicit pleiotropic responses in cultured human breast cancer cells, including effects on growth and differentiation. IL-1 β inhibits DNA synthesis and cell growth in several human breast cancer cell lines (Tsai and Gaffney, 1986; Paciotti and Tamarkin, 1988). Similarly, TPA also inhibits breast cancer cell growth, blocking entry into the G₁ phase and resulting in acquisition of a more differentiated phenotype. PGE₂ also inhibits proliferation in human breast cancer cells (Fentiman *et al.*, 1984). However, a mediatory role for PGE₂ during these anti-mitotic actions of IL-1 β and TPA is unknown.

Aromatase mediates the conversion of androstenedione to oestrone and may play an important role in regulating local oestrogen levels in breast tumours. Aromatase activity in breast cancer cells is stimulated by various growth factors following activation of the protein kinase A and C signalling pathways (Ryde *et al.*, 1992). Since PGE₂ itself is an agonist for the adenylate cyclase/cAMP system in breast cancer cells (Fentiman *et al.*, 1984), the potential also exists for aromatase regulation by this eicosanoid. Modulation of breast cancer cell aromatase by IL-1 β is unknown. However, this cytokine has been reported to stimulate aromatase in breast stromal fibroblasts (Reed *et al.*, 1992). In a comparative study with several human breast cancer cell lines, we have identified MDA MB 231 cells as having a relatively high capacity for arachidonate metabolism via the cyclo-oxygenase pathway (Schrey and Patel, 1995). This cell line also exhibits significant aromatase activity in comparison with other human breast cancer cell lines (A. Purohit, personal communication). In the present study, we have investigated a potential role for IL-1 β and protein kinase C in the regulation of arachidonate metabolism, aromatase activity and growth in MDA MB 231 cells. The possibility of an autocrine mechanism involving PGE₂ during the regulation of cell function by IL-1 β and TPA has also been examined.

METHODS

Cell culture

The cell line MDA MB 231 was obtained from the National Cell Bank (Porton Down, UK). Cells were grown in 25 cm²

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flasks and maintained in Eagle's minimal essential medium (EMEM) supplemented with glutamine (2 mM) and bicarbonate (4.5 mM) containing 20 mM HEPES (pH 7.4) and 5% FCS. In all experiments, MDA MB 231 cells were seeded into multiwell plates in EMEM containing 5% FCS, penicillin (100 units/ml), streptomycin (100 µg/ml) and amphotericin B (250 ng/ml).

For studies monitoring PGE₂ production or aromatase activity, cells were grown to approximately 70% confluence in 12- or 6-well plates, respectively, serum-starved for 4 hr and incubated for 24 hr in serum-free medium in the absence or presence of arachidonic acid (10 µM) and in the presence or absence of TPA and/or IL-1β or various other treatments (see figure legends for details). PGE₂ release into the medium was measured by radio-immunoassay (see below).

Cell growth

The effect of various treatments (see legends for details) on MDA MB 231 cell growth was monitored after 4 days of culture in EMEM containing 5% FCS. MDA MB 231 cells were seeded into 6-well plates at approximately 2×10^4 cells per well and allowed to plate down for 18 hr before treatments were added. Cell nuclei were counted by the Coulter principle following treatment with Zaponin (Coulter, Luton, UK). There were no significant changes in cell numbers in response to any treatments after 24 hr in either the studies measuring PGE₂ production or those measuring aromatase activity.

Measurement of PGE₂

Release of PGE₂ into the culture media was measured using a specific radio-immunoassay as previously described (Kelly *et al.*, 1986). The anti-serum employed was raised to the methyl oxime of PGE₂. At the end of each experiment, aliquots of the culture media were derivatised at room temperature overnight with equal volumes of methyloximating reagent. No agents employed in this study interfered with the assay at the concentrations used. The sensitivity of the assay is 43 nmol/ml⁻¹. The percentage cross-reactivity of the antibody with various derivatised arachidonate metabolites has been determined as follows: PGE₂, 100; 15-keto PGE₂, 0.25; PGF_{2α}, <0.02; PGB₂, 0.2; PGD₂, 0.02; TXB₂, <0.02; 6-keto PGF_{1α}, 0.02. The mean intra-assay coefficient of variation was 6.2%. All samples within the same experimental group were measured in the same assay.

Measurement of aromatase activity

Aromatase activity was determined in intact MDA MB 231 cell monolayers under serum-free conditions by measuring the release of ³H₂O from [1β-³H]androstenedione as previously described (Newton *et al.*, 1986) during an 18 hr assay period subsequent to the 24 hr incubation in the presence of various treatments (see legends for details). Unless otherwise stated, dexamethasone (100 nM) was also present during this assay period. Assay incubations were initiated by addition of 4.4×10^5 dpm [1β-³H]androstenedione (21.5 Ci/mmol) to each well. Released radioactivity was determined after repeated extraction of the medium with ether and subsequent treatment with dextran-coated charcoal. Activity is expressed as femtomoles of product formed per 10⁶ cells.

Expression of data

All values are presented as the mean ± SD from individual representative experiments each performed in triplicate and each conducted on at least 3 separate occasions. Statistical significance of differences between experimental groups was determined by analysis of variance followed by unpaired Student's *t* test.

RESULTS

Synergistic stimulation of arachidonic acid metabolism in MDA MB 231 cells by IL-1β and TPA

Neither IL-1β nor TPA alone or in combination had any effect on PGE₂ production in MDA MB 231 cells in the

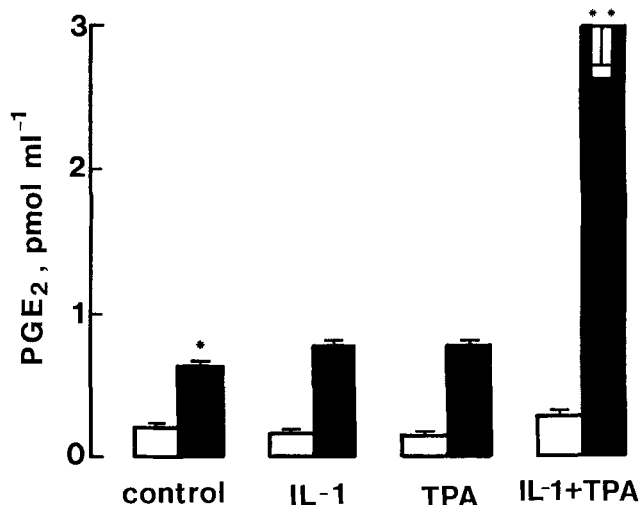


FIGURE 1 – Effect of arachidonic acid, IL-1β and TPA on PGE₂ production in MDA MB 231 cells. Cells were incubated without (open bars) or with (solid bars) arachidonic acid (10 µM) in the absence or presence of IL-1β (0.6 nM), TPA (10 nM) or IL-1β plus TPA for 24 hr. PGE₂ was measured as described. **p* < 0.001 for stimulation by arachidonic acid alone. ***p* < 0.001 for stimulation by IL-1β plus TPA in the presence of arachidonic acid.

absence of exogenous prostaglandin substrate. Constitutive cyclo-oxygenase activity was apparent as evidenced by a 3-fold increase in immunoreactive PGE₂ production in the presence of 10 µM arachidonic acid (Fig. 1). IL-1β alone failed to induce PGE₂ production in the presence of arachidonate in this (Fig. 1) and all subsequent experiments. However, in the presence of TPA, a synergistic increase in PGE₂ in response to IL-1β was consistently observed in the presence of substrate (Fig. 1). This synergism was dose-related with respect to both the cytokine and the phorbol ester (Fig. 2), with maximal responses to TPA being observed at 100 nM (Fig. 2*b*). This response to TPA in both the presence and absence of IL-1β was biphasic, with both responses being reduced by 50% at 1 µM TPA compared with 100 nM TPA (Fig. 2*b*). Bryostatin 1, another protein kinase C agonist, also stimulated a modest increase in PGE₂ production, which was further enhanced in the presence of IL-1β, albeit to a much smaller degree compared with the TPA responses (Table I).

Cholera toxin, which is known to stimulate cAMP accumulation in MDA MB 231 cells, had no effect on PGE₂ production (Table I). Indomethacin, a well-known potent inhibitor of the cyclo-oxygenase pathway, and dexamethasone, a potent suppressor of cyclo-oxygenase-2 mRNA and protein induction (Crofford *et al.*, 1994), both completely suppressed the increases in arachidonate metabolism to PGE₂ in response to IL-1β and TPA (Table II).

The mechanistic basis for the presently observed synergism between the cytokine and phorbol ester is unknown and could potentially occur at several different loci. Since a modulatory role for protein kinase C has been previously implicated in the regulation of cytokine action *via* changes in the expression of the IL-1β receptor in some cell types (Aksamit *et al.*, 1993), we have conducted a receptor-binding study with ¹²⁵I-IL-1β to investigate such a role in MDA MB 231 cells. However, although specific binding to MDA MB 231 cells was detectable, this was very low in both the absence and presence of TPA, precluding any accurate Scatchard analysis of the data (data not shown).

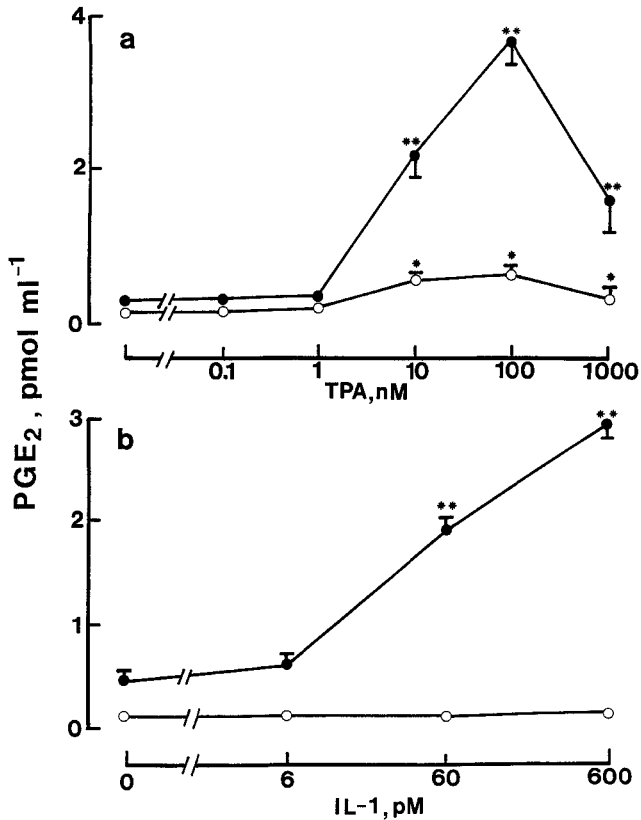


FIGURE 2 – PGE₂ production in MDA MB 231 cells as a function of (a) TPA concentration and (b) IL-1β concentration. Cells were incubated for 24 hr in the presence of arachidonic acid (10 μM) in (a) without (open circles) or with (solid circles) IL-1β (0.6 nM) with increasing concentrations of TPA or in (b) without (open circles) or with (solid circles) TPA (10 nM) with increasing concentrations of IL-1β. **p* < 0.01 for stimulation by TPA. ***p* < 0.01 for stimulation by IL-1β in the presence of TPA.

TABLE I – EFFECT OF TPA, BRYOSTATIN 1, CHOLERA TOXIN AND IL-1β ON PGE₂ PRODUCTION IN MDA MB 231 CELLS

	PGE ₂ production fmol ml ⁻¹			
	Control	TPA	Bryostatin 1	Cholera toxin
No addition	428 ± 88	1,159 ± 125 ¹	680 ± 23 ¹	442 ± 17
IL-1β	535 ± 77	6,411 ± 332 ²	1,000 ± 94 ²	558 ± 96

MDA MB 231 cells were incubated in the presence of arachidonic acid (10 μM) with or without TPA (10 nM), bryostatin 1 (30 nM), cholera toxin (50 ng/ml) or IL-1β (0.6 nM) for 24 hr. PGE₂ was measured by radio-immunoassay as described.

¹*p* < 0.01 for stimulation above control values. ²*p* < 0.01 for enhancement of TPA and bryostatin 1 responses in the presence of IL-1β.

Inhibition of cell growth by IL-1β and TPA

The effect of IL-1β, TPA and arachidonate on MDA MB 231 cell growth after 4 days of culture is shown in Table III. TPA (10 nM) consistently inhibited cell growth in the presence and absence of exogenous arachidonate, while arachidonate alone also inhibited growth in 2 of 3 experiments (Table III). IL-1β alone or in the presence of arachidonate had no consistent overall effect on cell proliferation but significantly inhibited growth in the presence of TPA in 7 of 8 instances (Table III). Inhibition of arachidonate metabolism by dexamethasone or indomethacin had no effect on the anti-mitotic actions of TPA or IL-1β in the presence of TPA during 4 days

TABLE II – INHIBITION OF PGE₂ PRODUCTION BY INDOMETHACIN AND DEXAMETHASONE

	PGE ₂ production fmol ml ⁻¹	
	Basal	IL-1β plus TPA
Control	946 ± 221	5,717 ± 1,235 ¹
Indomethacin	249 ± 68	280 ± 71 ²
Control	1,043 ± 28	6,380 ± 1,161 ¹
Dexamethasone	1,227 ± 57	1,380 ± 142 ²

MDA MB 231 cells were incubated for 24 hr in the presence or absence of IL-1β (0.6 nM), TPA (10 nM), indomethacin (1 μM) or dexamethasone (100 nM) in medium containing arachidonic acid (10 μM). PGE₂ was measured by radio-immunoassay.

¹*p* < 0.01 for stimulation above control values. ²*p* < 0.01 for inhibition of the responses to IL-1β plus TPA.

TABLE III – INHIBITION OF MDA MB 231 CELL GROWTH BY IL-1β AND TPA

Experiment	Arachidonic acid	Percent inhibition of cell growth			
		Control	IL-1β	TPA	IL-1β plus TPA
1	-	0	6 ± 1	58 ± 4 ¹	76 ± 23
	+	4 ± 1	5 ± 1	59 ± 5 ¹	81 ± 9 ²
2	-	0	3 ± 5	60 ± 4 ¹	75 ± 2 ³
	+	30 ± 6 ¹	40 ± 9	79 ± 2 ¹	84 ± 2 ²
3	-	0	7 ± 2	71 ± 2 ¹	84 ± 1 ³
	+	19 ± 3 ¹	23 ± 2	82 ± 1 ¹	89 ± 1 ³
4	-	0	14 ± 4	46 ± 2 ¹	61 ± 4 ³
	+	0	13 ± 12	22 ± 5 ¹	47 ± 2 ³

MDA MB 231 cell growth was monitored after 4 days of culture in the presence or absence of arachidonic acid (10 μM), IL-1β (0.6 nM) and TPA (10 nM). Cells were then counted, and the reduction in number following treatments is expressed as a percent inhibition of control values determined in the absence of arachidonic acid.

¹*p* < 0.01 for significant inhibition of cell growth compared with control growth in the absence or presence of arachidonic acid where appropriate. ²*p* < 0.05 and ³*p* < 0.01 for inhibition of growth by IL-1β in the presence of TPA compared with values for TPA alone.

of MDA MB 231 cell growth (Fig. 3). Dexamethasone had a small stimulatory effect on growth (Fig. 3). Treatment of MDA MB 231 cells with exogenous PGE₂ (2.8 × 10⁻⁵ M) throughout 4 days of culture had no effect on cell numbers at the end of this growth period (data not shown).

Effect of IL-1β and TPA on aromatase activity

One potential functional role for PGE₂ which has not been previously investigated in breast cancer cells is the regulation of aromatase. In this respect, activation of protein kinase A signalling, which has been previously implicated during PGE₂ action in breast cancer cells, also stimulates aromatase activity in various cell types (Evans *et al.*, 1987), including breast cancer cells (Ryde *et al.*, 1992). Furthermore, since protein kinase C activation and IL-1 action may influence breast cancer cell aromatase, a mediatory role for PGE₂ during cytokine and phorbol ester action in MDA MB 231 cells has also been investigated.

In the absence of dexamethasone, aromatase activity in MDA MB 231 cells remained unresponsive to a variety of agonists, including cholera toxin and PGE₂ (Fig. 4). Dexamethasone alone consistently stimulated aromatase (Fig. 4). This aromatase activity in the presence of dexamethasone was further enhanced by cholera toxin and PGE₂ (Fig. 4).

The aromatase inhibitor 4-hydroxyandrostenedione completely suppressed these responses, consistent with a primary role for aromatase in the measured increases in tritiated water production (Fig. 4). The protein kinase C agonist TPA also

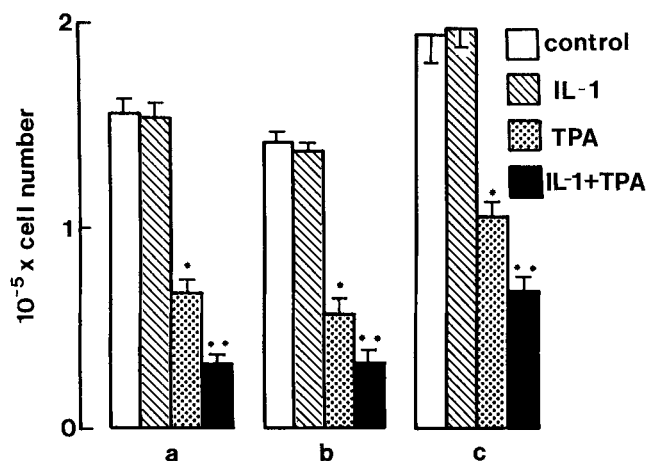


FIGURE 3 – Effect of indomethacin and dexamethasone on the inhibition of MDA MB 231 cell growth by TPA and IL-1 β . MDA MB 231 cells were grown in medium containing 5% FCS and 10 μ M arachidonic acid for 4 days in the absence (a) or presence (b) of indomethacin (1 μ M) or (c) dexamethasone (100 nM) and in the absence or presence of 0.6 nM IL-1 β , 10 nM TPA or IL-1 β and TPA together. Cell numbers were measured at the end of this incubation period. * p < 0.01 for inhibition of cell growth by TPA compared with respective control values. ** p < 0.02 for inhibition of cell growth by IL-1 β in the presence of TPA compared with appropriate TPA values.

stimulated aromatase in the presence of dexamethasone (Fig. 5). These data thus support a potential role for protein kinase C, protein kinase A and PGE₂ in aromatase regulation in MDA MB 231 cells. IL-1 β alone had no effect on aromatase activity but significantly increased the response to TPA (Fig. 5). These responses were further enhanced in the presence of arachidonic acid (Fig. 5). However, indomethacin had no effect on these stimulatory responses, which were again abolished in the presence of 4-hydroxyandrostenedione (Fig. 5). The permissive effect of dexamethasone on all of the aromatase responses described above were observed when dexamethasone was present during the last 18 hr of incubation, *i.e.*, during the measurement of [³H]androstenedione metabolism. When MDA MB 231 cells were pre-treated with dexamethasone, although the stimulatory effect of the corticosteroid remained apparent (Fig. 6), aromatase stimulation by the combined treatment of IL-1 β and TPA in both the presence and absence of arachidonic acid was completely suppressed (Fig. 6).

DISCUSSION

Malignant breast tumour tissue contains high levels of PGE₂ (Karmali *et al.*, 1983), the regulation and consequences of which are poorly understood. In the present study, we have examined the effect of IL-1 β and TPA on PGE₂ production in the human breast cancer cell line MDA MB 231. A potential role for PGE₂ as an autocrine mediator during the regulation of cell growth and aromatase activity by the cytokine and phorbol ester has also been investigated.

Both constitutive and TPA-inducible cyclo-oxygenase activity was apparent in MDA MB 231 cells as measured by increases in PGE₂ production in the presence of exogenous substrate. While impotent alone, IL-1 β consistently stimulated PGE₂ production in the presence of TPA. Similarly, a synergistic increase in PGE₂ was also observed with another protein kinase C agonist, bryostatin 1. The biphasic nature of the PGE₂ dose-response to TPA may reflect down-regulation

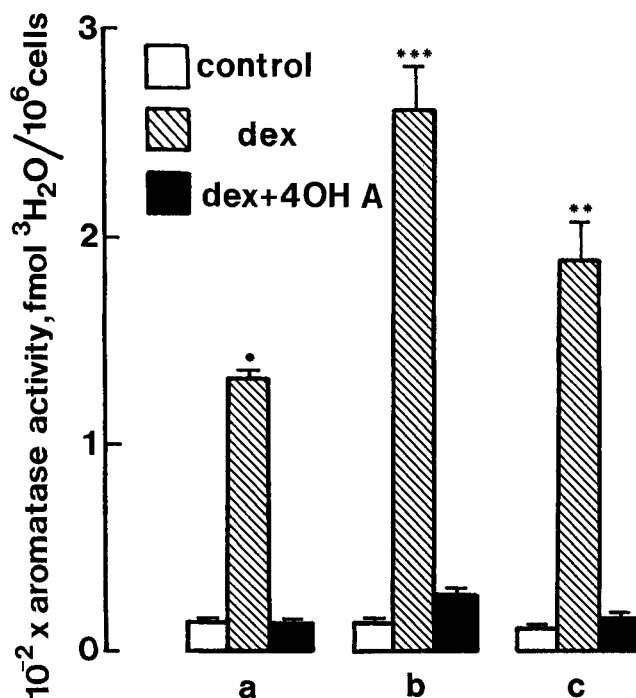


FIGURE 4 – Effect of dexamethasone, PGE₂ and cholera toxin on aromatase activity in MDA MB 231 cells. Cells were pre-treated for 24 hr (a) without or (b) with cholera toxin (50 ng/ml) or (c) PGE₂ (10 μ M) prior to measuring aromatase activity (see “Methods” for details) during a subsequent incubation with aromatase substrate in the absence (open bars) or presence of 0.1 μ M dexamethasone (hatched bars) or dexamethasone plus 1 μ M 4-hydroxyandrostenedione (solid bars). * p < 0.001 for stimulation by dexamethasone alone. ** p < 0.05 and *** p < 0.01 for stimulation by PGE₂ and cholera toxin in the presence of dexamethasone.

of protein kinase C occurring at higher doses of the phorbol ester. The permissive effect of exogenous arachidonate on the observed potentiation between IL-1 β and TPA suggests that any synergistic interaction between the signalling pathways employed by these agonists in MDA MB 231 cells does not involve enhancement of substrate availability, thus obviating a role for phospholipase A₂. Previous studies in fibroblasts have indicated a stimulatory role for protein kinase C in the up-regulation of IL-1 binding sites and expression of IL-1 receptor mRNA (Aksamit *et al.*, 1993). We have been unable to establish whether such a mechanism sensitises MDA MB 231 cells to the cytokine. Other workers have similarly failed to detect IL-1-binding sites on MDA MB 231 cells (Paciotti and Tamarkin, 1988) despite reports of functional responses to the cytokine in these cells (Tsai and Gaffney, 1986). The nature of the IL-1 receptor involved in the presently described actions of the cytokine in MDA MB 231 cells thus remains unknown but may comprise the low-level, high-affinity receptor previously detected in other cell types.

Stimulation of PGE₂ production in MDA MB 231 cells by IL-1 β and TPA and inhibition of this response by dexamethasone is consistent with a role for cyclo-oxygenase-2, the enzyme activity and mRNA expression of which are induced by the cytokine and phorbol ester and inhibited by glucocorticoids in several cell types, including synovial fibroblasts (Crofford *et al.*, 1994). Whether the increase in PGE₂ production in MDA MB 231 cells in response to IL-1 β and protein kinase C activation partly reflects the pathophysiological mechanisms driving the elevated PGE₂ levels which are associated with aggressive malignant breast tumours is unknown. Nonetheless, infiltra-

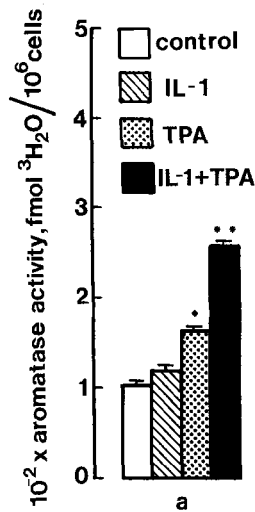


FIGURE 5—Effect of IL-1 β , TPA and arachidonic acid on aromatase activity. MDA MB 231 cells were pre-treated for 24 hr without or with 0.6 nM IL-1 β , 10 nM TPA or IL-1 β plus TPA in (a) the absence or (b) the presence of 10 μ M arachidonic acid or (c) arachidonic acid plus 1 μ M indomethacin or (d) arachidonic acid plus 1 μ M 4-hydroxyandrostenedione. Aromatase activity was monitored during a subsequent incubation in the presence of 0.1 μ M dexamethasone. * p < 0.01 for stimulation by TPA alone. ** p < 0.01 for stimulation by IL-1 β in the presence of TPA compared with TPA alone. *** p < 0.01 for enhancement of the TPA and TPA plus IL-1 β responses by arachidonic acid.

tion of cytokine-producing host-immune cells and high protein kinase C activity are also characteristic of malignant breast tumours. Indeed, the MDA MB 231 cell line also exhibits an invasive phenotype and possesses high protein kinase C activity.

PGE₂ may act as an autocrine and/or paracrine modulator of cell growth and function on both cancer cells and stromal components within breast tumours (Patel and Schrey, 1992; Schrey and Patel, 1994). In the present study, stimulation of arachidonic acid metabolism *via* the cyclo-oxygenase pathway by TPA and IL-1 β was accompanied by an inhibition of cell growth and an increase in aromatase activity. As with PGE₂ production, MDA MB 231 cells remained unresponsive to IL-1 β alone, whereas the cytokine further enhanced growth inhibition and aromatase activity in response to TPA. The mechanistic basis for this permissive effect of TPA on IL-1 β action is again unknown but may reflect protein kinase C-mediated up-regulation of the IL-1 receptor as already mentioned. Whether down-regulation by TPA of a PKC-mediated mitogenic pathway is partly responsible for the anti-proliferative effects of the phorbol ester in the present study is unknown. However, a mediatory role for the cyclo-oxygenase pathway during growth regulation and aromatase activation by the cytokine and phorbol ester appears to be ruled out since indomethacin did not prevent these actions. A role for prostaglandins during growth inhibition of MCF-7 cells by IL-1 has been similarly discounted (Tsai and Gaffney, 1986). Previous studies with MDA MB 231 cells have reported either no effect of IL-1 alone on cell growth (Paciotti and Tamarkin, 1988) or a small (6%) inhibitory effect (Tsai and Gaffney, 1986). Interestingly, a role for the lipoxigenase pathway has been implicated during the stimulation of MDA MB 231 cell growth by ω -6 fatty acids (Rose and Connolly, 1990).

Stimulation of aromatase activity in MDA MB 231 cells by TPA and IL-1 β was further enhanced in the presence of exogenous arachidonic acid and could be the consequence of metabolism *via* an alternative route such as the lipoxigenase

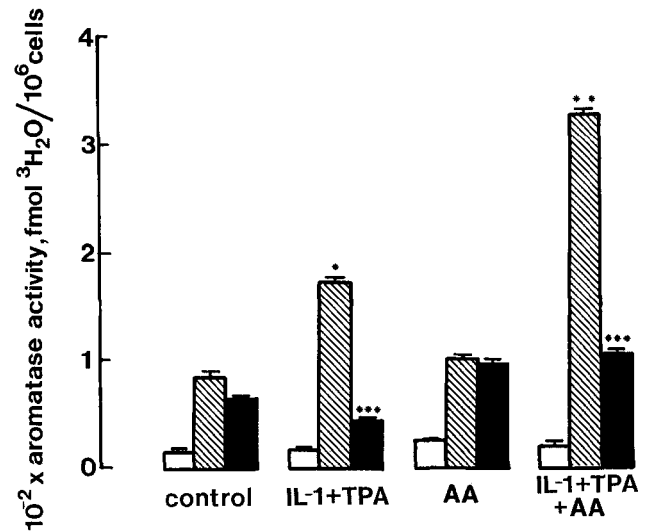


FIGURE 6—Effect of dexamethasone pre-treatment on aromatase stimulation by IL-1 β and TPA. MDA MB 231 cells were pre-treated for 24 hr without or with 0.6 nM IL-1 β plus 10 nM TPA or 10 μ M arachidonic acid or IL-1 β plus TPA plus arachidonic acid. Aromatase activity was measured during a subsequent 18 hr incubation (see "Methods"). Dexamethasone (100 nM) was either absent throughout the entire time period (open bars), present during the last 18 hr—*i.e.*, during the aromatase assay only—(hatched bars) or present for the whole 42 hr (solid bars). * p < 0.001 for stimulation by IL-1 β plus TPA. ** p < 0.01 for enhancement of IL-1 β plus TPA response by arachidonic acid. *** p < 0.001 for inhibition of IL-1 β plus TPA responses by dexamethasone pre-treatment.

pathway or possibly due to the direct modulation of protein kinase C activity and/or other signalling pathways for the fatty acid *per se* (Graber *et al.*, 1994). A potential role for PGE₂ as an independent regulator of breast cancer cell aromatase is, however, not ruled out since PGE₂ alone did indeed stimulate aromatase activity in MDA MB 231 cells. A role for this eicosanoid has also been implicated in the regulation of aromatase in ovarian granulosa cells (Wang *et al.*, 1982). Previous studies have indicated a role for cAMP in aromatase regulation in various cell types (Wang *et al.*, 1982; Evans *et al.*, 1987), including the breast cancer cell lines MCF-7 and T47D (Ryde *et al.*, 1992). Since the adenylate cyclase activator cholera toxin also stimulated aromatase in MDA MB 231 cells and PGE₂ is known to stimulate cAMP production in human breast cancer cells (Fentiman *et al.*, 1984), a potential mediatory role for this signalling pathway during aromatase activation by PGE₂ is also possible.

We report an interactive role for IL-1 β , arachidonic acid and protein kinase C in the regulation of aromatase activity in human breast cancer cells. The permissive effect of dexamethasone on this action further exemplifies the multifactorial control of aromatase found in other cell types (Evans *et al.*, 1987; Reed *et al.*, 1992; Ryde *et al.*, 1992). Indeed, the cellular mechanisms concerned with the control of aromatase expression are complex, involving tissue-specific regulation by alternative promoters (Simpson *et al.*, 1994). Speculation on the nature of the interactive synergism between glucocorticoids, IL-1 β and TPA during the regulation of aromatase activity in MDA MB 231 cells must obviously await further definitive investigation. Dexamethasone has been long known to stimulate aromatase activity in adipose stromal cells but only in the presence of a serum factor(s) (Simpson *et al.*, 1981). Interestingly, MDA MB 231 cells have been shown to produce a factor resembling this serum-derived aromatase-stimulating activity (Schmidt and Loffler, 1994). Hence, the possibility that such a

factor may participate as an autocrine mediator during aromatase stimulation should also be considered. Regulation of aromatase by dexamethasone in MDA MB 231 cells was biphasic. Thus, when cells were exposed to dexamethasone during IL-1 β and TPA treatment, aromatase stimulation by the glucocorticoid remained evident, whilst the response to the cytokine and phorbol ester was abolished. Dexamethasone treatment thus appears to impair signal transduction by IL-1 and TPA but presumably not as a consequence of cyclooxygenase inhibition since indomethacin had no effect on aromatase stimulation. In addition to cyclo-oxygenase, glucocorticoids are known to down-regulate the expression of a variety of other IL-1 β - and TPA-responsive genes. Whether modulation of aromatase activity by dexamethasone in MDA MB 231 cells similarly operates at the level of gene expression remains to be investigated.

In summary, our study in MDA MB 231 cells describes a synergistic interaction between IL-1 β and TPA, leading to an increase in arachidonic acid metabolism, a stimulation of aromatase activity and an inhibition of cell growth. PGE₂ does

not appear to mediate these actions on aromatase activity or growth. A potential autocrine role for PGE₂ in MDA MB 231 cells is nonetheless demonstrable, as evidenced by an increase in aromatase activity in response to exogenous PGE₂. Alternatively, MDA MB 231 cells may represent a paracrine source for PGE₂ in the control of tumour cell growth and function. Finally, notwithstanding the hormone-independent nature of MDA MB 231 cells, given the heterogeneity of breast tumours, aromatase activity in these cells may constitute a paracrine mechanism regulating hormone-dependent growth in neighbouring cells within the tumour. Indeed, breast tumour stromal cell aromatase is also thought to play a role in the control of local oestrogen levels in breast cancer (Reed *et al.*, 1993).

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REFERENCES

- AKSAMIT, T.R., MONICK, M.M. and HUNNINGHAKE, G.W., Protein kinase C modulates the amount of IL-1 receptor mRNA in human lung fibroblasts. *J. Immunol.*, **151**, 284–290 (1993).
- CROFFORD, L.J., WILDER, R.L., RISTIMÄKI, A.P., SANO, H., REMMERS, E.F., EPPS, H.R. and HLA, T., Cyclooxygenase-1 and -2 expression in rheumatoid synovial tissues. Effects of interleukin-1 β , phorbol ester and corticosteroids. *J. clin. Invest.*, **93**, 1095–1101 (1994).
- EVANS, C.T., CORBIN, J., SAUNDERS, C.T., MERRILL, J.C., SIMPSON, E.R. and MENDELSON, C.R., Regulation of estrogen biosynthesis in human adipose stromal cells. Effects of cAMP, epidermal growth factor and phorbol esters on the synthesis of aromatase cytochrome P450. *J. Biol. Chem.*, **262**, 6914–6920 (1987).
- FENTIMAN, I.S., DUHIG, T., GRIFFITHS, A.B. and TAYLOR-PAPADIMITRIOU, J., Cyclic AMP inhibits the growth of human breast cancer cells in defined medium. *Mol. Biol. Med.*, **2**, 81–88 (1984).
- FULTON, A.M., Effects of indomethacin on the growth of cultured mammary tumors. *Int. J. Cancer*, **33**, 375–379 (1984).
- FULTON, A.M. and HEPPNER, G.H., Relationship of prostaglandin E and natural killer sensitivity to metastatic potential in murine mammary adenocarcinoma. *Cancer Res.*, **45**, 4779–4784 (1985).
- FULTON, A.M., ZHANG, S.-Z. and CHONG, Y.C., Role of the prostaglandin E₂ receptor in mammary tumor metastasis. *Cancer Res.*, **51**, 2047–2050 (1991).
- GRABER, R., SUMIDA, C. and NUNEZ, E.A., Fatty acids and cell signal transduction. *J. Lipid Mediat. Cell. Signal.*, **9**, 91–116 (1994).
- HLA, T. and NEILSON, K., Human cyclooxygenase-2 cDNA. *Proc. nat. Acad. Sci. (Wash.)*, **89**, 7384–7388 (1992).
- KARMALI, R.A., WELT, S., THALER, H.T. and LEFEVRE, F., Prostaglandins in breast cancer. Relationship to disease stage and hormone status. *Brit. J. Cancer*, **48**, 689–696 (1983).
- KELLY, R.W., DEAM, S., CAMERON, M.J. and SEAMARK, R.F., Measurement by radio-immunoassay of prostaglandins as their methyl oximes. *Prostaglandins Leukotrienes essent. Fatty Acids*, **24**, 1–14 (1986).
- NEWTON, C.J., SAMUEL, D.L. and JAMES, V.H.T., Aromatase activity and concentrations of cortisol progesterone and testosterone in breast and abdominal tissue. *J. Steroid Biochem.*, **24**, 1033–1039 (1986).
- PACIOTTI, G.F. and TAMARKIN, L., Interleukin-1 directly regulates hormone-dependent human breast cancer cell proliferation *in vitro*. *Mol. Endocrinol.*, **2**, 459–464 (1988).
- PATEL, K.V. and SCHREY, M.P., Inhibition of DNA synthesis and growth by bradykinin in human breast stromal cells. Evidence for independent roles for B₁ and B₂ receptors in the respective control of cell growth and phospholipid hydrolysis. *Cancer Res.*, **52**, 334–340 (1992).
- REED, M.J., COLDHAM, N.G., PATEL, S.R., GHILCHIK, M.W. and JAMES, V.H.T., Interleukin-1 and interleukin-6 in breast cyst fluid: their role in regulating aromatase activity in breast cancer cells. *J. Endocrinol.*, **132**, R5–R8 (1992).
- REED, M.J., TOPPING, L., COLDHAM, N.G., PUROHIT, A., GHILCHIK, M.W. and JAMES, V.H.T., Control of aromatase activity in breast cancer cells: the role of cytokines and growth factors. *J. Steroid Biochem. mol. Biol.*, **44**, 589–596 (1993).
- ROSE, D.P. and CONNOLLY, J.M., Effects of fatty acids and inhibitors of eicosanoid synthesis on the growth of a human breast cancer cell line in culture. *Cancer Res.*, **50**, 7139–7144 (1990).
- RYDE, C.M., NICHOLLS, J.E. and DOWSETT, M., Steroid and growth factor modulation of aromatase activity in MCF-7 and T47D breast carcinoma cell lines. *Cancer Res.*, **52**, 1411–1415 (1992).
- SCHMIDT, M. and LOFFLER, G., The human breast cancer cell line MDA MB 231 produces an aromatase stimulating activity. *Europ. J. Cell. Biol.*, **63**, 96–101 (1994).
- SCHREY, M.P. and PATEL, K.V., Role of regulatory peptides in the control of breast cancer cell growth and function. *Endocrine-Related Cancer*, **1**(3), 41–70 (1994).
- SCHREY, M.P. and PATEL, K.V., Prostaglandin E₂ production and metabolism in human breast cancer cells and breast fibroblasts. Regulation by inflammatory mediators. *Brit. J. Cancer*, **72**, 1412–1419 (1995).
- SIMPSON, E.R., ACKERMAN, G.E., SMITH, M.E. and MENDELSON, C.R., Estrogen formation in stromal cells of adipose tissue of women: induction by glucocorticoids. *Proc. nat. Acad. Sci. (Wash.)*, **78**, 5690–5694 (1981).
- SIMPSON, E.R., MAHENDROO, M.S., MEANS, G.D., KILGORE, M.W., HINSELWOOD, M.M., GRAHAM-LORENCE, S., AMARNEH, B., ITO, Y., FISHER, C.R., MICHAEL, M.D., MENDELSON, C.R. and BULUN, S.E., Aromatase cytochrome P450, the enzyme responsible for estrogen biosynthesis. *Endocr. Rev.*, **15**, 342–355 (1994).
- TSAI, S.-C. and GAFFNEY, E.V., Inhibition of cell proliferation by interleukin-1 derived from monocytic leukemia cells. *Cancer Res.*, **46**, 1471–1477 (1986).
- WANG, C., HSUEH, A.J. and ERICKSON, G.F., The role of cyclic AMP in the induction of oestrogen and progesterin synthesis in cultured granulosa cells. *Mol. cell. Endocrinol.*, **25**, 73–83 (1982).
- WATSON, J. and CHUAH, S.Y., Technique for the primary culture of human breast cancer cells and measurement of their prostaglandin secretion. *Clin. Sci.*, **83**, 347–352 (1992).