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Prostaglandins E_1 and E_2 interact with prostaglandin $F_{2\alpha}$ to regulate initiation of DNA replication and cell division in Swiss 3T3 cells

(lag phase/insulin/2-deoxyglucose uptake/wound healing/transformation)

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ABSTRACT **Prostaglandin (PG) E_1 or E_2 added at 2–1,000 ng/ml to quiescent cultures of Swiss 3T3 cells synergistically enhanced the rate of initiation of DNA replication stimulated by $PGF_{2\alpha}$ alone or with insulin. Neither PGD_2 nor $PGF_{1\alpha}$ had any effect with $PGF_{2\alpha}$. An increase in the rate of entry into S phase also occurred when PGE_1 or PGE_2 was added 8 or 15 hr after addition of $PGF_{2\alpha}$. However, adding PGE_1 and PGE_2 together with $PGF_{2\alpha}$ did not further enhance the synergistic effect observed with PGE_1 or PGE_2 separately. The synergistic effect was also observed in stimulation of 2-deoxyglucose uptake but not in early changes of intracellular levels of cAMP. These results may be relevant in understanding the control of fibroblastic proliferation in wound healing and may provide an alternative mechanism for oncogenic transformation.**

An important property of normal animal cells is their ability to regulate the frequency of initiation of DNA replication and cell division in response to changes in the physiological and architectural requirements of the organism (1–4). Among the growth-promoting substances and hormones involved in modulating the regulatory mechanisms are prostaglandins (PGs) of the E and F series, which are synthesized and released by various mammalian cell types in response to different pathological and physiological changes (4–6). Different human cancers (7–9), chemically and virally induced tumors in animals (10, 11), and cultured human and animal cancer cells (12, 13) produce high levels of PGE_1, PGE_2, and $PGF_{2\alpha}$. Likewise, BALB/c 3T3 cells transformed by oncogenic DNA viruses exhibit increased rates of synthesis of PGE_1, PGE_2, and $PGF_{2\alpha}$ compared with their normal counterparts (14). In normal tissue, wound healing is accomplished mainly by the proliferation of fibroblasts (15). The primary response to injury is the aggregation of platelets in capillaries at the edge of the wound (16) and it has been shown *in vitro* that $PGF_{2\alpha}$ and PGE_2 are released on aggregation (17). However, a causal relationship between release of the PGs and fibroblastic proliferation *in vivo* has not yet been established.

It has been shown in resting confluent cultures of Swiss 3T3 and other fibroblastic cells that $PGF_{2\alpha}$ can stimulate chromosomal DNA replication and cell division (18–20). In particular, Swiss 3T3 cells have provided a useful model system to study how $PGF_{2\alpha}$ alone or with modulating hormones regulates cell proliferation (4). Here, we show that PGE_1 and PGE_2 added at physiological concentrations to quiescent Swiss 3T3 cells synergistically enhance the rate of initiation of DNA synthesis stimulated by $PGF_{2\alpha}$. The relevance of these results for understanding wound-healing as well as uncontrolled growth of transformed or oncogenic cells producing PGs is discussed.

MATERIALS AND METHODS

Cell Cultures. Swiss mouse 3T3 cells (21) were propagated at 37°C in 10% CO_2/90% air in Dulbecco's modified Eagle's medium/10% fetal calf serum containing streptomycin at 100 μg/ml and penicillin at 100 units/ml (4).

Assay for Initiation of DNA Synthesis and Determination of Rate Constant for Entry into S Phase. Swiss 3T3 cells were plated at 1.5×10^5 per 35-mm dish in 2 ml of Dulbecco's modified Eagle's medium/6% fetal calf serum supplemented with low molecular weight nutrients as before (4, 20). Three days after seeding, the cells were given fresh medium supplemented as before and then allowed to become confluent and quiescent for 3 to 4 days. PGs were dissolved in ethanol and diluted so that the final concentration of ethanol in the conditioned medium was <0.05%. Cells were radioactively labeled for autoradiography by exposing the cultures to 1 μM [*methyl*-^{3}H]thymidine (3 μCi/ml; 1 Ci = 3.7×10^{10} becquerels)] from the time of additions until the times indicated in each experiment. Pairs of cultures were then processed for autoradiography (4). The rate constant (k) for entry into S phase and the duration of the lag phase were calculated as described before (4).

cAMP Measurement. For determination of total intracellular levels of cAMP, the cells were plated at 2.5×10^5 per 50-mm dish in 5 ml of culture medium as for the assay of DNA synthesis. Thirty or sixty minutes after additions, the culture medium was removed and cultures were rinsed twice within 10 sec with cold 140 mM NaCl/6.7 mM KCl/0.7 mM $CaCl_2$/0.5 mM $MgCl_2$/0.4 mM Na_2PO_4/25 mM Tris·HCl, pH 7.4. Then, cAMP was extracted by covering the cell monolayer with 1 ml of 0.1 M HCl in 95% ethanol at 4°C and scraping the cells from the dish. The dish was rinsed with 1 ml of the same solution, and the rinse was pooled with the cell extract. After 16 hr or longer, the samples were centrifuged at $1,060 \times g$ for 20 min at 4°C. The clear supernatants were evaporated to dryness on a 60°C water bath using an airstream. The dried extracts were suspended in radioimmunoassay buffer (New England Nuclear kit for determining cAMP by radioimmunoassay) and assayed in duplicate for cAMP (22). This method recovers at least 90% of the radioactive cAMP added to the samples at the time of addition of acidic ethanol. In addition, the cyclic nucleotide nature of the radioimmunoactivity was verified by cyclic nu-

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Abbreviations: PG, prostaglandin; PGD_2, prostaglandin D_2; PGE_1, prostaglandin E_1; PGE_2, prostaglandin E_2; $PGF_{1\alpha}$, prostaglandin $F_{1\alpha}$; $PGF_{2\alpha}$, prostaglandin $F_{2\alpha}$.

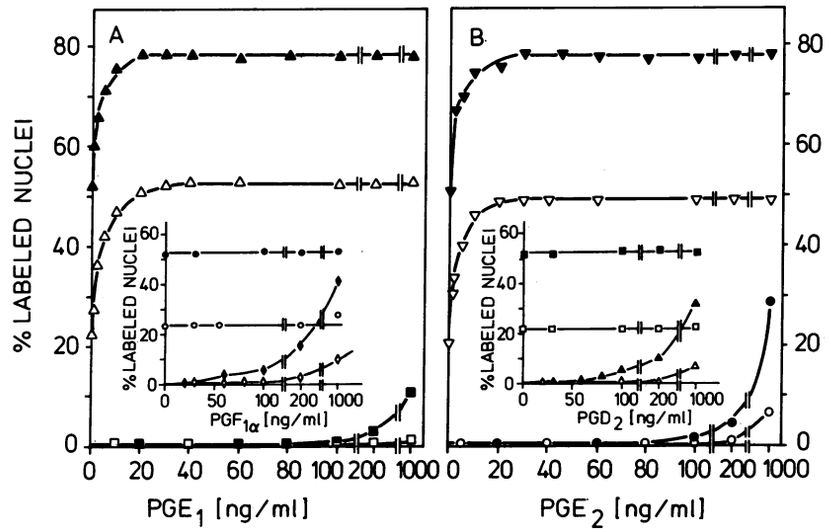

FIG. 1. Enhancement by PGE_1 (*A*) and PGE_2 (*B*) of initiation of DNA synthesis stimulated by $PGF_{2\alpha}$ (300 ng/ml) alone or with insulin (60 ng/ml). (*A*) △, $PGF_{2\alpha}/PGE_1$; ▲, $PGF_{2\alpha}$/insulin/PGE_1; □, PGE_1 only; ■, PGE_1/insulin. (*Inset*): ○, $PGF_{2\alpha}/PGF_{1\alpha}$; ●, $PGF_{2\alpha}$/insulin/$PGF_{1\alpha}$; ◇, $PGF_{1\alpha}$ only; ◆, $PGF_{1\alpha}$/insulin. (*B*) ▽, $PGF_{2\alpha}/PGE_2$; ▼, $PGF_{2\alpha}$/insulin/PGE_2; ○, PGE_2 only; ●, PGE_2/insulin. (*Inset*) □, $PGF_{2\alpha}/PGD_2$; ■, $PGF_{2\alpha}$/insulin/PGD_2; △, PGD_2 only; ▲, PGD_2/insulin. Cultures were exposed to [*methyl*-^{3}H]thymidine for 0–28 hr after additions and then processed for autoradiography.

cleotide phosphodiesterase treatment of control samples prior to radioimmunoassay (23). The protein pellets were dissolved in 100 μl of 0.4 M NaOH, and the mixtures were incubated at 30°C for 15 min and then diluted by adding 250 μl of water. Duplicate samples were analyzed for protein content according to Bradford (24).

Measurement of 2-Deoxyglucose Uptake. Uptake of 2-deoxy[^{3}H]glucose was determined in a 10-min pulse 6 hr after stimulation as described (25).

Materials. PGs were the gift of John Pike, Upjohn Company. Fatty acids, insulin, and thymidine were purchased from Sigma. [*methyl*-^{3}H]Thymidine (28 Ci/mmol) and 2-deoxy[^{3}H]glucose (22 Ci/mmol) were obtained from the Radiochemical Centre (Amersham, England). Antibodies and the ^{125}I-labeled cAMP tracer for determination of cAMP were purchased from New England Nuclear.

RESULTS

Interaction of $PGF_{2\alpha}$ with Other PGs: Effect on DNA Synthesis. $PGF_{2\alpha}$ added alone at a saturating concentration (300 ng/ml) to quiescent Swiss 3T3 cells stimulated the initiation of DNA synthesis, resulting in 22% labeled nuclei after 28 hr (Fig. 1). For other PGs, such as PGE_1, PGE_2, $PGF_{1\alpha}$, and PGD_2, which differ from $PGF_{2\alpha}$ only by a hydroxyl group or the Δ^5 double bond, much higher concentrations were required to even marginally stimulate DNA synthesis in these cells (Fig. 1). Insulin at a concentration that does not stimulate DNA synthesis (60 ng/ml) enhanced the stimulatory effect of $PGF_{2\alpha}$ to a labeling index of 50%. The stimulatory effects on the other four PGs were also enhanced by insulin; however, even at 1 μg/ml, the synergistic effects were less than that observed with $PGF_{2\alpha}$ at the lower concentration (Figs. 1 and 2).

PGE_1, at 2 ng–1 μg/ml, added to cells stimulated by $PGF_{2\alpha}$ (300 ng/ml) synergistically enhanced the labeling index by increasing it to 51% (Fig. 1*A*). Similarly, PGE_2, at the same concentration, increased the $PGF_{2\alpha}$-stimulated labeling index to 48% (Fig. 1*B*). The concentration of PGE_1 and PGE_2 giving maximal enhancement was ≈20 ng/ml. Insulin further enhanced the stimulation by $PGF_{2\alpha}$ with PGE_1 or PGE_2 to 78% (Fig. 1 *A* and *B*). The addition of PGE_1 (100 ng/ml) and PGE_2 (100 ng/ml) together to cells stimulated by $PGF_{2\alpha}$ (300 ng/ml) did not further increase the enhancement produced by either PGE_1 or PGE_2 separately (Table 1). The stimulation of DNA synthesis was also reflected in an increase in cell number 48 hr after additions (Table 1). Neither $PGF_{1\alpha}$ nor PGD_2 at concentrations up to 1 μg/ml had any effect on cells stimulated by $PGF_{2\alpha}$, either alone or with insulin (Fig. 1 *A* and *B Insets*). PGs A_1, A_2, B_1, and I_2 (prostacyclin), which alone show no stimulatory effect on DNA synthesis (up to 1 μg/ml) in these cells (26), likewise did not enhance the effect of $PGF_{2\alpha}$ (not shown).

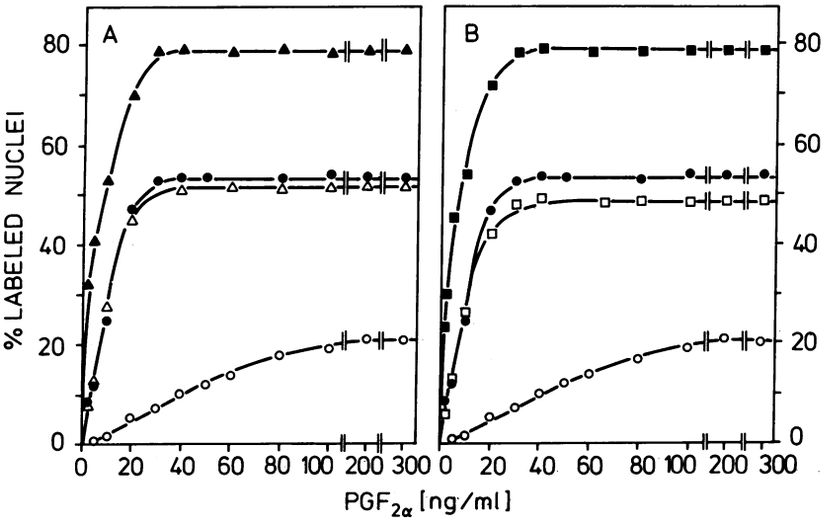

FIG. 2. Effect of various concentrations of $PGF_{2\alpha}$ alone or with PGE_1 (100 ng/ml) or PGE_2 (100 ng/ml) without or with insulin (60 ng/ml). (*A*) ○, $PGF_{2\alpha}$ only; ●, $PGF_{2\alpha}$/insulin; △, $PGF_{2\alpha}/PGE_1$; ▲, $PGF_{2\alpha}$/insulin/PGE_1. (*B*) ○, $PGF_{2\alpha}$ only; ●, $PGF_{2\alpha}$/insulin; □, $PGF_{2\alpha}/PGE_2$; ■, $PGF_{2\alpha}$/insulin/PGE_2. Cultures were exposed to [*methyl*-^{3}H]thymidine for 0–28 hr after additions and then processed for autoradiography.

Table 1. Interaction of PGs and fatty acids with $PGF_{2\alpha}$: Effect on labeling index and cell number

Addition(s)	Without insulin		With insulin	
	% labeled nuclei	Cells, no. × 10^{-5}	% labeled nuclei	Cells, no. × 10^{-5}
None	0.5	7.9	0.7	8.0
$PGF_{2\alpha}$	22.0	9.6	51.8	12.0
$PGF_{2\alpha}/PGE_1$	52.0	12.1	77.5	14.1
$PGF_{2\alpha}/PGE_2$	49.7	11.9	78.0	13.9
$PGF_{2\alpha}/PGF_{1\alpha}$	23.4	9.7	53.0	12.2
$PGF_{2\alpha}/PGD_2$	21.6	9.5	52.8	12.3
$PGF_{2\alpha}$/linoleic acid	21.7	—	51.4	—
$PGF_{2\alpha}$/oleic acid	21.9	—	51.2	—
$PGF_{2\alpha}$/arachidonic acid	22.0	—	50.9	—
$PGF_{2\alpha}/PGE_1/PGE_2$	53.9	12.9	79.0	14.3
PGE_1/PGE_2	1.8	—	3.5	—
10% fetal calf serum	95.0	17.1	—	—

$PGF_{2\alpha}$ and fatty acids were added at 300 ng/ml. Other PGs were added at 100 ng/ml. For determination of labeling index, cells were exposed to [*methyl*-^{3}H]thymidine for 0–28 hr after additions before they were processed for autoradiography. For determination of cell number, cells were plated at 1.5 × 10^5 per 50-mm dish as for the DNA synthesis assay, suspended 48 hr after additions with 0.05% trypsin, and then counted in isotonic buffer with a Coulter Counter.

Furthermore, linoleic acid, oleic acid, and arachidonic acid (a precursor of PGs) had no effect on DNA synthesis (Table 1).

Since physiological concentrations of PGs in normal tissues and body fluids are generally lower than the saturating concentrations for stimulating DNA synthesis in Swiss 3T3 cells, the synergistic effect of these PGs was studied at concentrations 1–10% of those found in various human tissues (27). At 3 ng/ml, neither $PGF_{2\alpha}$, PGE_1, nor PGE_2 alone had any stimulatory effect on quiescent cells within 28 hr (Fig. 1 and Table 2). $PGF_{2\alpha}$ together with either PGE_1 or PGE_2 had a very small effect (about 2% labeled nuclei). However, in the presence of a low concentration of insulin (6 ng/ml), this marginal stimulation was enhanced to 11%. For $PGF_{2\alpha}$, PGE_1, and PGE_2 each added alone at 6 ng/ml, only $PGF_{2\alpha}$ showed some stimulatory activity with insulin (Table 2). Addition of PGE_1 or PGE_2 together with $PGF_{2\alpha}$, all at 6 ng/ml, had a synergistic effect resulting in a labeling index of ≈8%, which was further enhanced by insulin to ≈23% within 28 hr. Thus, at these low concentrations, $PGF_{2\alpha}$ together with insulin and PGE_1 or PGE_2 stimulates quiescent confluent Swiss 3T3 cells to initiate DNA synthesis. Adding both PGE_1 and PGE_2 together with $PGF_{2\alpha}$, alone or with insulin, at these low concentrations did not further increase the effect produced by PGE_1 or PGE_2 alone (Table 2).

Table 2. Interaction of $PGF_{2\alpha}$ with PGE_1, PGE_2, and insulin at low concentrations

Addition(s)	% labeled nuclei	
	Without insulin	With insulin (6 ng/ml)
None	0.52 ± 0.33	0.51 ± 0.23
	Prostaglandins at 3 ng/ml	
PGE_1	0.51 ± 0.21	0.60 ± 0.14
PGE_2	0.49 ± 0.16	0.31 ± 0.12
$PGF_{2\alpha}$	1.04 ± 0.57	2.51 ± 0.41
$PGF_{2\alpha}/PGE_1$	2.24 ± 0.48	10.94 ± 1.50
$PGF_{2\alpha}/PGE_2$	1.68 ± 0.38	10.30 ± 1.46
$PGF_{2\alpha}/PGE_1/PGE_2$	2.60 ± 0.52	10.86 ± 1.30
	Prostaglandins at 6 ng/ml	
PGE_1	0.51 ± 0.14	0.48 ± 0.20
PGE_2	0.50 ± 0.19	0.53 ± 0.23
$PGF_{2\alpha}$	2.40 ± 0.86	6.55 ± 0.78
$PGF_{2\alpha}/PGE_1$	7.67 ± 1.27	23.20 ± 2.89
$PGF_{2\alpha}/PGE_2$	7.45 ± 0.87	21.14 ± 2.85
$PGF_{2\alpha}/PGE_1/PGE_2$	8.50 ± 1.40	23.33 ± 2.19

Labeling index was determined as in Table 1. Results are mean ± SD calculated from six different experiments. Each experiment was done in duplicate and an average of 1,200 cells were counted per dish.

Addition of either PGE_1 or PGE_2 at 100 ng/ml reduced the saturating concentration of $PGF_{2\alpha}$ required to maximally stimulate DNA synthesis from ≈200 to 30 ng/ml (Fig. 2). In the presence of insulin and PGE_1 or PGE_2, concentrations of $PGF_{2\alpha}$ as low as 2 ng/ml were sufficient to increase the labeling index to ≈30% after 28 hr.

Time-Dependent Regulation by PGs of Entry into S Phase. $PGF_{2\alpha}$ stimulates DNA synthesis by regulating two different parameters: (*i*) the length of the lag phase (≈15 hr) and (*ii*) the rate of initiation of DNA synthesis, which follows apparent first-order kinetics. The latter process is quantified by a rate constant k (4). How do PGE_1 and PGE_2 modulate the stimulation of DNA synthesis? The synergistic effect of PGE_1 and PGE_2 was due to a marked increase in the rate of entry into S without changing the length of the lag phase (Fig. 3 *A* and *B*). PGE_1 or PGE_2 can also be added 9 or 15 hr into the lag phase, but then the synergistic effect was less than that observed when either was added together with $PGF_{2\alpha}$. However, when PGE_1 or PGE_2 was added at 15 hr, the time required to increase the initial $PGF_{2\alpha}$-induced rate was ≈5 hr.

Insulin did not change the pattern of interaction of PGE_1 or PGE_2 with $PGF_{2\alpha}$, although it increased the rate of entry into S phase. Adding PGE_1 or PGE_2 9 hr after $PGF_{2\alpha}$ and insulin, however, resulted in the same rate constant as if PGE_1 or PGE_2 had been added with $PGF_{2\alpha}$ and insulin (Fig. 3 *C* and *D*). A similar enhancement was observed when PGE_1 or PGE_2 was added 15 hr after $PGF_{2\alpha}$ and insulin, yet 5 hr were required to increase the rate induced by $PGF_{2\alpha}$ and insulin.

Changes in Intracellular Levels of cAMP and 2-Deoxyglucose Uptake. In some cellular systems a predominant effect of PGE_1 is to increase the intracellular levels of cAMP. $PGF_{2\alpha}$ induces the protein synthesis-dependent phase of 2-deoxyglucose uptake in Swiss 3T3 cells (4, 25). A question as to the mechanism by which these PGs act is whether the synergistic effect of PGE_1 and PGE_2 with $PGF_{2\alpha}$ on initiation of DNA synthesis is expressed through these two biochemical events. Neither $PGF_{2\alpha}$, insulin, nor $PGF_{2\alpha}$ and insulin together increased the intracellular cAMP levels measured after 30 and 60 min, and fetal calf serum transiently reduced the cAMP level (Table 3). PGE_1,

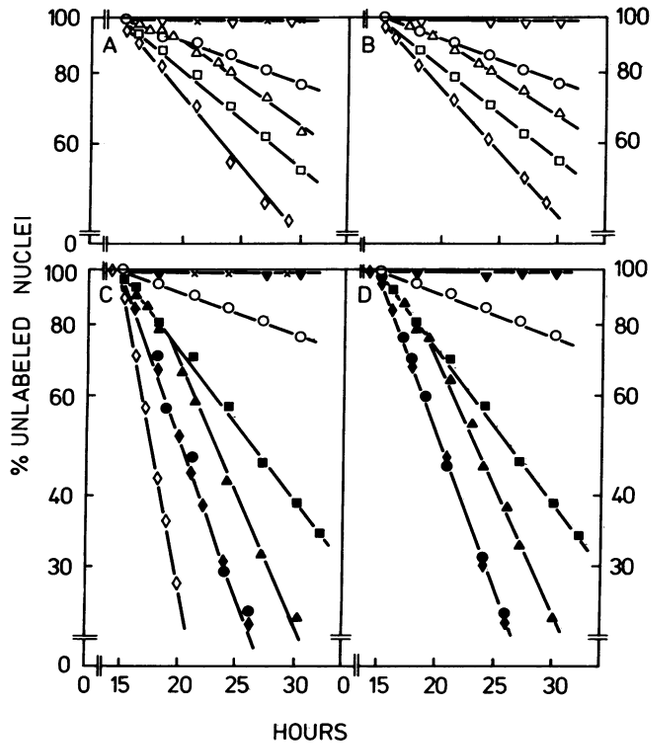

FIG. 3. Kinetics of entry into S phase stimulated by $PGF_{2\alpha}$ (300 ng/ml) alone or with PGE_1 (100 ng/ml) or PGE_2 (100 ng/ml) without or with insulin (60 ng/ml). (*A*) x, no additions (k = 0.0005/hr); ▽, PGE_1 only (k = 0.0006/hr); ○, $PGF_{2\alpha}$ only (k = 0.016/hr); $PGF_{2\alpha}$ with PGE_1 added at 0 hr (◇; k = 0.055/hr), 9 hr (□; k = 0.039/hr), and 15 hr (△; k = 0.033/hr). (*B*) ▽, PGE_2 only (k = 0.0006/hr); ○, $PGF_{2\alpha}$ only (k = 0.016/hr); $PGF_{2\alpha}$ with PGE_2 added at 0 hr (◇; k = 0.051/hr), 9 hr (□; k = 0.037/hr), and 15 hr (△; k = 0.032/hr). (*C*) x, insulin (k = 0.0006/hr); ▼, PGE_1/insulin (k = 0.0005/hr); ○, $PGF_{2\alpha}$ only (k = 0.016/hr); ■, $PGF_{2\alpha}$/insulin (k = 0.061/hr); ◇, serum only (k = 0.246/hr); $PGF_{2\alpha}$/insulin with PGE_1 added at 0 hr (◆; k = 0.13/hr), 9 hr (■; k = 0.13/hr), and 15 hr (▲; k = 0.12/hr). (*D*) ▼, PGE_2 only (k = 0.0006/hr); ○, $PGF_{2\alpha}$ only (k = 0.016/hr); ■, $PGF_{2\alpha}$/insulin (k = 0.061/hr); $PGF_{2\alpha}$/insulin with PGE_2 added at 0 hr (◆; k = 0.12/hr), 9 hr (●; k = 0.12/hr), and 15 hr (▲; k = 0.11/hr). In all cases, the lag phase was 14.5 hr long; [*methyl*-^{3}H]thymidine was present from the initial addition until the times indicated.

alone or with insulin, increased the cAMP level after 30 min about 5-fold and, after 60 min, the cAMP level decreased to values near to control. In contrast, PGE_2 stimulated only a marginal increase after 30 min. Thus, PGE_1 and PGE_2 differ in that, at these concentrations, only PGE_1 increased the intracellular cAMP level and that transiently. When added with $PGF_{2\alpha}$ or $PGF_{2\alpha}$ and insulin, PGE_1 stimulated a transient increase in cAMP levels similar to that observed with PGE_1 alone. Adding PGE_2 with $PGF_{2\alpha}$ or $PGF_{2\alpha}$ and insulin did not increase cAMP levels as compared with the effect of $PGF_{2\alpha}$. Thus, there appears to be no correlation of intracellular cAMP level in the first 60 min of the lag phase and the rate of initiation of DNA synthesis.

The synergistic interaction of $PGF_{2\alpha}$ and insulin is observed in the stimulation of the protein synthesis-dependent phase of 2-deoxyglucose uptake measured 6 hr after additions (4, 25) (Table 3). Both PGE_1 and PGE_2 had only marginal effects on 2-deoxyglucose uptake, and only an additive effect was observed together with insulin (Table 3). In contrast, both PGE_1 and PGE_2 had synergistic effects with $PGF_{2\alpha}$ on 2-deoxyglucose uptake that were further enhanced by insulin. As observed for DNA synthesis, adding PGE_1 and PGE_2 together with $PGF_{2\alpha}$ did not result in further enhancement of 2-deoxyglucose uptake (Table 3).

Table 3. Changes in intracellular cAMP levels and 2-deoxyglucose uptake on addition of PGs

Addition(s)	cAMP, pmol/mg of protein		2-Deoxyglucose uptake, (pmol/min)/mg of protein (5 hr)
	30 min	60 min	
None	18.5	19.5	8.8
Insulin	19.0	16.9	21.8
$PGF_{2\alpha}$	17.9	12.1	39.6
$PGF_{2\alpha}$/insulin	20.3	14.3	106.2
PGE_1	91.7	30.4	12.6
PGE_1/insulin	96.8	33.0	26.5
PGE_2	23.2	16.4	10.5
PGE_2/insulin	29.2	11.8	28.2
$PGF_{2\alpha}/PGE_1$	95.5	50.8	95.8
$PGF_{2\alpha}/PGE_1$/insulin	111.4	30.4	169.6
$PGF_{2\alpha}/PGE_2$	9.4	17.5	85.0
$PGF_{2\alpha}/PGE_2$/insulin	15.7	19.0	158.7
$PGF_{2\alpha}/PGE_1/PGE_2$	ND	ND	96.0
Fetal calf serum	5.3	13.6	312.0

Additions were made to quiescent Swiss 3T3 cells cultured as described for initiation of DNA synthesis. Concentrations used were insulin, 60 ng/ml; $PGF_{2\alpha}$, 300 ng/ml; PGE_1, 100 ng/ml; PGE_2, 100 ng/ml; fetal calf serum, 10%. Experimental values were within 10% of the mean. ND, not determined.

DISCUSSION

The observations that, in Swiss 3T3 cells, $PGF_{2\alpha}$ alone induces a low rate of initiation of DNA synthesis and the stimulatory effect of $PGF_{2\alpha}$ can be modulated by hormones such as insulin (which alone does not stimulate DNA synthesis) are compatible with the present view that *in vivo* the growth of normal cells is regulated by low concentrations of various hormones and growth-stimulating factors. Here, we have shown that another class of PGs, PGE_1 and PGE_2, which like $PGF_{2\alpha}$ are produced by numerous mammalian cell types, can synergistically interact with $PGF_{2\alpha}$ in stimulating initiation of DNA synthesis. Together with another ubiquitous hormone, insulin, very low concentrations of PGs are sufficient to stimulate ≈25% of the cells to initiate DNA synthesis within 28 hr. PGE_1 and PGE_2, like insulin, approximately double the rate of $PGF_{2\alpha}$-induced entry into S phase when added at any time of the lag phase; each requires ≈5 hr to increase the rate when supplemented at the end of the lag phase (Fig. 3; ref. 4). However, since insulin increases the PGE_1 or PGE_2 enhancement of the $PGF_{2\alpha}$ effect, PGE_1 and PGE_2 are likely to act through different mechanisms than insulin. Furthermore, PGE_1 and PGE_2 together with $PGF_{2\alpha}$ did not further enhance the synergy produced by either PGE_1 or PGE_2 separately. This indicates that PGE_1 and PGE_2 probably act through a common mechanism with an event involved in regulating the rate of initiation of DNA synthesis.

The synergistic interaction between $PGF_{2\alpha}$ and PGE_1 or PGE_2 is also seen in the protein synthesis-dependent phase of 2-deoxyglucose uptake. The stimulation of 2-deoxyglucose uptake by $PGF_{2\alpha}$, as well as its enhancement by PGE_1, PGE_2, or insulin, was blocked by actinomycin and cycloheximide (not shown), indicating that these processes are dependent on mRNA and protein synthesis. This suggests that the synergy observed on DNA synthesis may be expressed through a protein synthesis-dependent event. But it is not yet known whether the synergistic effect observed on 2-deoxyglucose uptake is expressed on the same or on a different pathway from that leading to entry into S phase.

The observation that PGE_1, but not PGE_2, stimulates a transient increase of intracellular cAMP levels is supported by similar results in other clones of Swiss 3T3 cells (ref. 6; unpublished results) as well as in BALB/c 3T3 cells (28). Likewise, PGE_2 is less effective than PGE_1 in stimulating adenylate cyclase in Swiss 3T3 cells (29). The synergistic effects of PGE_1 and PGE_2 with $PGF_{2\alpha}$ on DNA synthesis are, therefore, unlikely to be modulated by this event. Furthermore, $PGF_{2\alpha}$ alone or with insulin does not increase intracellular cAMP levels (Table 2), and 1–100 μM 8-bromo-cAMP had no effect on DNA synthesis stimulated by $PGF_{2\alpha}$ and insulin (data not shown). In contrast, high concentrations of PGE_1 (40 μg/ml), which increase intracellular cAMP to levels similar to those observed at low concentrations, or 8-bromo-cAMP at 1 mM inhibited the stimulation of DNA synthesis (ref. 4; data not shown). Thus, there appears to be no correlation between the early transient change in intracellular cAMP level and the regulation of rate of entry into S phase by PGs in Swiss 3T3 cells. This is in agreement with a recent report that, under different culture conditions, insulin, epidermal growth factor, and fibroblast-derived growth factor, which stimulated DNA synthesis, did not increase cAMP levels by themselves (30).

The observation that both PGE_1 and PGE_2 act synergistically with $PGF_{2\alpha}$ suggests that $PGF_{2\alpha}$ acts through different mechanisms than PGE_1 and PGE_2 and that $PGF_{2\alpha}$ interacts with a receptor that is distinct from that with which PGE_1 and PGE_2 interact. Indeed, different receptors for $PGF_{2\alpha}$ and PGE have been demonstrated on bovine corpora lutea cells (6). Furthermore, PGE receptors have high and low affinity sites, which preferably bind PGE_1 in a fibroblastic cell line (6). From the results with cAMP and 2-deoxyglucose, it is not apparent whether, in Swiss 3T3 cells, PGE_1 and PGE_2 act through different receptors or share a common receptor with different binding affinities.

The synergy between PGs may be an important mechanism involved in growth control of normal fibroblastic cells and uncontrolled growth of certain transformed cells. Normal fibroblastic growth leads to the formation of connective tissue, which, in the wound-healing process, results in a scar (15). Wounding leads to platelet aggregation on the blood vessel wall (16). Within 5 min after experimental induction of platelet aggregation by thrombin, $PGF_{2\alpha}$ and PGE_2 are released to concentrations of $\approx$10 and 35 ng/ml, respectively (17). The experiments presented in this paper show that these concentrations are high enough to stimulate proliferation and migration of fibroblasts (unpublished results) derived from tissue adjacent to a wound (15). In the presence of other hormones such as insulin this stimulation could be synergistically enhanced.

It has been postulated that transformed cells proliferate independently of serum factors because they are constitutively stimulated by polypeptide growth factors produced by themselves (31, 32). Since a number of cancer and transformed cells show a high production of PGs (5, 14), this may provide an alternative mechanism to bypass the normal controls of cell proliferation. Our results provide the framework for a hypothesis to study the role of PGs in the control of proliferation of normal and transformed cells.

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