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Transforming growth factor β1, insulin and prostaglandin E_1 enhance prostaglandin $F_{2\alpha}$ mitogenic action in Swiss 3T3 cells via separate events

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Abstract Transforming growth factor β1 (TGFβ1) had no mitogenic effect in Swiss 3T3 cells, but could increase prostaglandin $F_{2\alpha}$ (PGF2α)-induced DNA synthesis. Insulin, but not prostaglandin E_1 (PGE1), further enhanced PGF2α action at low TGFβ1 concentrations. TGFβ1 also acted concertedly with the protein kinase C (PKC) activator 1-oleoyl-2-acetylglycerol to induce mitogenesis. Thus, it appears that TGFβ1 and insulin act via separate signals, while TGFβ1 and PGE1 might share a common pathway not involving TGFβ1-mediated prostaglandin synthesis. These results suggest that TGFβ1 might elicit various signalling mechanisms to enhance PGF2α-triggered events.

Key words: Insulin; TGFβ1; Protein kinase C; DNA synthesis

1. Introduction

Mammalian cell division involves coordinated events resulting in the onset of DNA synthesis and mitosis [1,2]. Upon a variety of mitogenic stimuli, normal cells generate a complex signalling network to ensure the regulation of such phenomena [1–3]. Transforming growth factor β (TGFβ), a superfamily of closely related 25 kDa polypeptides, can either inhibit or induce cell division depending on the particular cell type [4–6]. Most TGFβ target cells possess three specific receptor molecules [5,7]. Upon TGFβ binding, these receptors undergo complex interactions, resulting in arrest of cell division as well as other cellular effects [5,7]. Whether the early TGFβ signals inducing cell cycle progression occur via such TGFβ receptors is not yet understood [5].

In confluent resting Swiss 3T3 cells, TGFβ is not mitogenic, but does enhance bombesin-induced DNA synthesis [8]. In these cells, prostaglandin $F_{2\alpha}$ (PGF2α) and bombesin trigger protein kinase C (PKC) and tyrosine kinase (TK) activation [9–11]. These PGF2α-dependent kinases can be independently activated with no mitogenic response. Nevertheless, such concerted kinase action is likely to be a PGF2α requirement to stimulate DNA synthesis [10,11]. Both insulin, prostaglandin E_1 (PGE1), and the combination of the two, potentiate PGF2α-mediated growth response [9].

Such findings pose two basic questions for the understanding of TGFβ action. The first deals with whether insulin, PGE1 and TGFβ act via separate processes to enhance PGF2α-induced DNA synthesis. The second concerns whether TGFβ-elicited events can interact with some PGF2α-dependent ones resulting in mitogenesis.

Here we show that TGFβ1 potentiates the PGF2α-mediated mitogenic response. Such action reflects an enhancement of the ability of cells to initiate DNA synthesis. At low TGFβ1 concentrations, insulin, but not PGE1, increased PGF2α action. In contrast, at high TGFβ1 concentrations insulin further increased PGF2α-induced glucose uptake. We also reveal that TGFβ1 can elicit signals complementary to some PGF2α-mediated events involved in the induction of DNA synthesis. TGFβ1 and insulin also exert concerted action, since only in combination could they induce mitogenesis. These results suggest that insulin, TGFβ1 and PGE1 enhance the action of PGF2α by triggering different events.

2. Materials and methods

2.1. Cell culture and DNA synthesis assay

Swiss mouse 3T3 cells [12] growth and DNA synthesis autoradiographic assay were carried out as described before [1]. To avoid the effect of albumin as a TGFβ1 carrier, TGFβ1 was dissolved at (10 μg/ml) in 1.0 mM CHAPS buffer and then further diluted in serum-free culture medium. CHAPS has no effect upon DNA synthesis.

2.2. Measurement of 2-deoxyglucose uptake

Cells were plated as for the DNA synthesis assay. Uptake assays were performed as previously described [13].

2.3. Materials

Recombinant TGFβ1 was purchased from R&D Minneapolis. Other chemicals were from Sigma Chemical Company. Methyl [^{3}H]thymidine (18 Ci/mmol) and 2-[1,2-3H]deoxyglucose (30 Ci/mmol) were obtained from New England Nuclear and American Radiolabeled Chemicals, respectively.

3. Results

3.1. TGFβ1 enhancement of PGF2α-induced DNA synthesis

The effects of TGFβ1 upon PGF2α-mediated mitogenesis in confluent, resting Swiss 3T3 cells is shown in Fig. 1. Stimulation by 40 and 300 ng/ml PGF2α induced initiation of DNA synthesis in 15% and 30% of cells, respectively. The addition of 0.05–1.0 ng/ml TGFβ1 enhanced PGF2α action. TGFβ1 at a concentration of 0.3 ng/ml in the presence of 300 ng/ml PGF2α, increased the number of cells in S phase up to 80%. At 40 ng/ml PGF2α, 1.0 ng/ml TGFβ1 was required to attain a similar effect (Fig. 1A). TGFβ1 at 0.8 ng/ml also reduced the amount of PGF2α required to induce a maximal response (Fig. 1B).

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Abbreviations: OAG, 1-oleoyl-2-acetylglycerol; PGE1, prostaglandin E_1; PGF2α, prostaglandin $F_{2\alpha}$; PKC, protein kinase C; TGFβ1, transforming growth factor β1.

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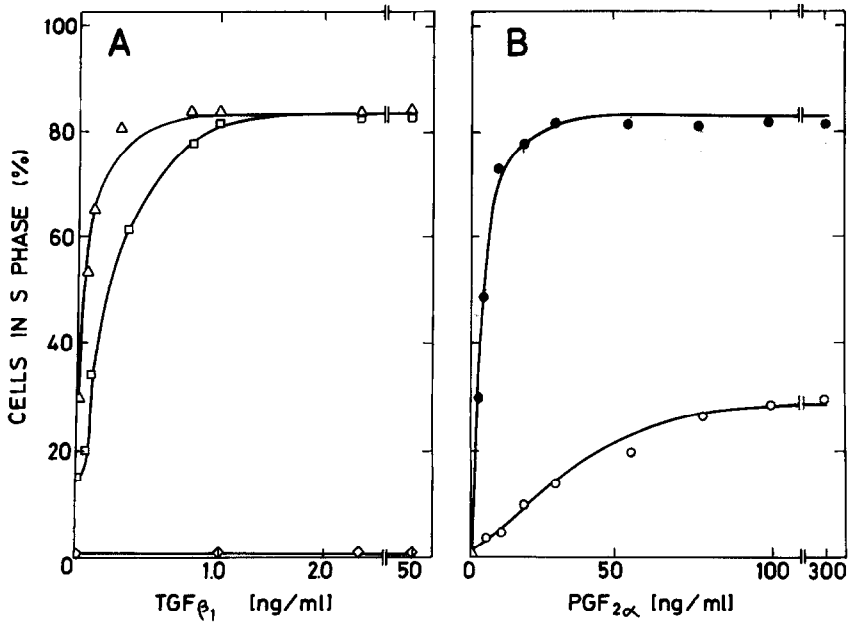

Fig. 1. (A) TGFβ1 dose–response of PGF2α-induced DNA synthesis. Additions were as indicated: (◇) TGFβ1, (□) PGF2α 40 ng/ml plus TGFβ1 and (△) PGF2α 300 ng/ml plus TGFβ1. (B) The effect of TGFβ1 on PGF2α dose-dependent response: (○) PGF2α, (●) PGF2α plus TGFβ1 (1.0 ng/ml). DNA synthesis was determined after 28 h of stimulation as indicated in section 2.1.

3.2. *TGFβ1, insulin and PGE1 enhance PGF2α-induced DNA synthesis via different events*

TGFβ1 action on PGF2α-induced DNA synthesis, with or without insulin or PGE1, are shown in Fig. 2. Concentrations of TGFβ1 of 0.05–1.0 ng/ml enhanced the PGF2α response. Insulin could further increase this effect only at TGFβ1 values of 0.05–0.2 ng/ml, but PGE1 failed to do so (Fig. 2A,B). In contrast, PGE1, insulin or the addition of both, raised PGF2α activity (Fig. 2B, insert). Neither TGFβ1, insulin, PGE1, nor the combination of TGFβ1 with PGE1 induced DNA synthesis. However, TGFβ1 plus insulin, or both along with PGE1, triggered mitogenesis (Fig. 2B). Indomethacin, an inhibitor of prostaglandin synthesis, did not block TGFβ1 potentiation of the PGF2α mitogenic action (not shown).

3.3. *TGFβ1 action complements PKC-dependent mitogenic events*

1-Oleoyl-2-acetylglycerol (OAG) (100 μg/ml), a permeable diacylglycerol analogue and PKC activator [11,14,15], did not induce DNA synthesis, but in the presence of TGFβ1 could trigger such a phenomenon (Table 1). Either insulin, PGE1 or both together acted in concert with OAG to induce mitogenesis [15]; yet insulin, though not PGE1, enhanced the actions of TGFβ1 and OAG on the stimulation of DNA synthesis (Table 1).

3.4. *Insulin and TGFβ1 increase PGF2α-induced DOG uptake*

The effects of TGFβ1, insulin and PGF2α on 2-deoxyglucose (DOG) uptake induction [13] after 6 h are shown in Table 2. Both TGFβ1 (0.8 ng/ml) and PGF2α (300 ng/ml) increased glucose uptake fivefold. Such effects could be blocked at least partially by cycloheximide, a protein synthesis inhibitor, while insulin-induced uptake remained unchanged. Both TGFβ1 and insulin were able to enhance the PGF2α-induced uptake. However, the addition of both insulin and PGF2α at these concentrations of TGFβ resulted in a further potentiated effect. Both actions could be blocked by cycloheximide (Table 2).

4. Discussion

Much evidence supports the fact that TGFβ can either inhibit or stimulate mammalian cell division [4–6]. TGFβ and a BSC-1 cell inhibitor, both identical molecules, enhance the mitogenic action of bombesin in Swiss 3T3 cells [8]. From the similarity between the action of TGFβ and insulin, it has been inferred that both enhance bombesin-induced mitogenesis via a common signalling mechanism [8].

Our findings reveal that in these cells, insulin and TGFβ1 elicit separate events, increasing PGF2α-mediated induction of mitogenesis and glucose uptake. Low, but not high, concentrations of TGFβ1 allow insulin to further potentiate the PGF2α-induced growth response. Such an effect might be reflecting a TGFβ1-dependent low signal threshold, requiring an insulin-triggered signal for full enhancement. In contrast, high concen-

Table 1
Complementary mitogenic induction by insulin, PGE1, OAG and/or TGFβ1

Additions	% labeled nuclei	
	− insulin	+ insulin
None	0.5	0.7
OAG	0.5	35.1
TGFβ1	0.8	10.0
PGE1	0.9	0.7
OAG+TGFβ1	61.0	78.0
OAG+PGE1	20.0	58.0
OAG+PGE1+TGFβ1	63.0	80.0

Insulin (50 ng/ml), OAG (100 μg/ml), PGE1 (100 ng/ml) and/or TGFβ1 (0.8 ng/ml) were added for 28 h. Induction of DNA synthesis was determined as in Fig. 1.

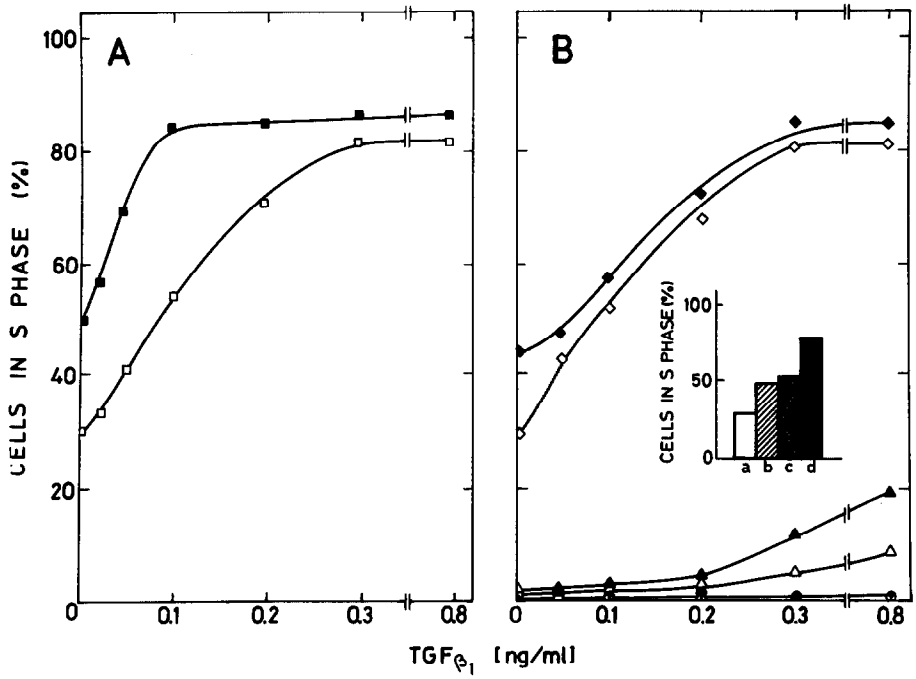

Fig. 2. Insulin and PGE1 action on PGF2α-mediated mitogenesis at various concentration of TGFβ1. Cells were exposed to PGF2α (300 ng/ml) with or without TGFβ1, insulin (50 ng/ml) and/or PGE1 (100 ng/ml). In A: (□) PGF2α plus TGFβ1, (■) PGF2α plus insulin and TGFβ1. In B: (◇) PGF2α plus TGFβ1, (◆) PGF2α plus PGE1 and TGFβ1, (○) TGFβ1, (△) TGFβ1 plus insulin, (●) TGFβ1 plus PGE1, (▲) TGFβ1 plus insulin and PGE1. Insert: a, PGF2α; b, PGF2α plus insulin; c, PGF2α plus PGE1; d, PGF2α plus PGE1 and insulin. Cells were labeled as in Fig. 1.

trations of TGFβ1, might either raise such a threshold or trigger other signal(s), thereby fully potentiating PGF2α-induced mitogenesis. Nevertheless, under the latter conditions, insulin can still enhance glucose uptake.

Further differences between the actions of TGFβ1 and insulin are supported by the fact that neither TGFβ1 nor insulin are mitogenic by themselves, but together can induce DNA synthesis. Moreover, the addition of TGFβ1 in combination with PGE1 failed to do so, yet PGE1 enhance TGFβ1 action in the presence of insulin. In contrast to insulin, PGE1 added together with PGF2α at low TGFβ1 concentrations, cannot cause further mitogenic enhancement. Such results suggest that TGFβ1 and PGE1 might be eliciting a common event. TGFβ1 action does not involve PGE1 synthesis since indomethacin, which blocks PGE1 formation, did not impair the enhancing action of TGFβ1 [16,17]. Nevertheless, TGFβ1- and PGE1-dependent events are not completely identical, since insulin induces mitogenesis in the presence of TGFβ1 but not PGE1.

Table 2
Effect of TGFβ1 and insulin upon PGF2α-induced 2-DOG uptake

Additions	2-DOG uptake (pmol/min/mg of protein)	
	− Cx	+ Cx
None	66	100
TGFβ1	343	190
Insulin	210	280
PGF2α	327	130
PGF2α+insulin	882	225
PGF2α+TGFβ1	1852	240
PGF2α+insulin+TGFβ1	2804	436

Additions were as follows: insulin (100 ng/ml), PGF2α (300 ng/ml and TGFβ1 (0.8 ng/ml). Upon 6 h of stimulation cells were labeled for 10 min with 2.5 μCi of 2-[1,2-^{3}H]deoxyglucose (50 μM) as indicated in section 2.2.

TGFβ1 signalling processes also differ from those elicited by PGF2α in Swiss 3T3 cells. PGF2α rapidly triggers increases in cellular contents of inositol 1,4,5 triphosphate, DAG, and Ca^{2+} mobilization, as well as PKC and TK activation [9,11,18]. In contrast, TGFβ1 neither elicits phosphoinositide metabolism nor increases Ca^{2+} fluxes [8]. However, TGFβ1 displays concerted action with some PGF2α-dependent events. In effect, DAG can cause PKC and TK activation [11], but only in combination with TGFβ1 is mitogenesis observed. This effect of TGFβ1 is potentiated by insulin but not by PGE1, though both of the latter have mitogenic effects in the presence of DAG [15].

Such actions of TGFβ1 and PGF2α on cell division might be relevant to two types of proliferative events. Both molecules are released by platelets in wound-healing processes, and thus possibly, stimulate the fibroblastic multiplication involved in tissue repair [19,20]. Some cancerous and transformed cells exhibit increased TGF and prostaglandin synthesis and release, causing unrestricted division [6,21]. To understand how TGFβ- and PGF2α-triggered signals lead to either one of these growth states is our research endeavour.

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References

[1] Jimenez de Asua, L., Richmond, K.M.V. and Otto, A.M. (1981) Proc. Natl. Acad. Sci. USA 78, 1004–1008.

[2] Rozengurt, E. (1986) Science 234, 161–166.
[3] Pardee, A.B. (1989) Science 246, 603–608.
[4] Massague, J. (1990) Annu. Rev. Cell Biol. 6, 597–641.
[5] Massague, J., Atisano, L. and Wrana, J.L. (1994) Trends Cell Biol. 4, 172–178.
[6] Sporn, M.B., Roberts, A., Wakefield, L.M. and Assoian, R. (1986) Science 233, 532–534.
[7] Lin, H.Y. and Lodish, H.F. (1993) Trends Cell Biol. 3, 14–19.
[8] Brown, K.D. and Holley, R.W. (1987) Proc. Natl. Acad. Sci. USA 84, 3747–3747.
[9] Goin, M., Pignataro, O. and Jimenez de Asua, L. (1993) FEBS Lett. 316, 68–72.
[10] Zachary, I., Gil, J., Lehmann, W., Sinnett-Smith, J. and Rozengurth, E. (1991) Proc. Natl. Acad. Sci. USA 88, 4577–4581.
[11] Jimenez de Asua, L. and Goin, M. (1994) in: Eicosanoids and Other Bioactive Lipids in Cancer Inflammation and Radiation Injury, vol. 3 (Hoon, K.V., Marnett, L.J. and Nigam, S. eds.) pp. 525–532, Kluwer, Norwell, MA.
[12] Todaro, G.J. and Green, H. (1963) J. Cell Biol. 17, 299–313.
[13] Jimenez de Asua, L., O'Farrell, M.K., Bennet, D., Clingan, D. and Rudland, P.S. (1977) Nature 265, 151–153.
[14] Rozengurt, E., Rodriguez-Peña, A., Commbs, M. and Sinnet-Smith, J. (1984) Proc. Natl. Acad. Sci. USA 81, 5748–5752.
[15] Jimenez de Asua, L., Estevez, A.G. and Goin, M. (1992) in: Eicosanoids and Other Bioactive Lipids in Cancer, Inflammation and Radiation Injury, vol. 2 (S. Nigam, K.V. Honn, L.J. Marnett and T. Walden eds. pp. 213–218), Kluwer, Norwell, MA.
[16] Tashjian, A.H., Voelkel, E.F., Lazzaro, B., Singer, F.R., Rorbets, A.B., Deryck, R., Mingler, N.E. and Levine, L. (1985) Proc. Natl. Acad. Sci. USA 82, 4535–4538.
[17] Diaz, A., Varga, J. and Jimenez, S.A. (1989) J. Biol. Chem. 764–11554–11557.
[18] Corps, A.N., Cheek, T.R., Moreton, R.B., Berridge, M.J. and Brown, K.D. (1989) Cell Reg. 1, 75–86.
[19] Roberts, A.B. and Sporn, M.B. (1993) Growth Factors 8, 1–7.
[20] Hamberg, M., Svenson, J. and Sammuelson, B. (1974) Proc. Natl. Acad. Sci. USA 71, 3824–3828.
[21] Karmali, R.D., Horrobin, D.F., Menezes, J. and Patel, P. (1979) Pharmacol Res. Commun. 11, 69–75.